Late Quaternary Changes in the Westerly Winds over the Southern Ocean: the sub-Antarctic peatland record and response

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

Cover Illustration – Microphotographs of testate amoebae shells found in the population at Kampkoppie (Marion Island, sub-Antarctica). From left to right: *Argynnia dentistoma, Certesella certesi, Argynnia caudata*, *Arcella arenaria* and *Apodera vas.*

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Abstract

The Southern Ocean plays a crucial role in modulating global atmospheric CO_2 concentrations by acting as a large carbon sink. Upwelling driven by surface winds brings sequestered carbon from deep-water reservoirs to the ocean surface where it can saturate the potential sink and de-gas directly to the atmosphere. Specifically, the intensity and latitudinal position of the Southern Hemisphere Westerly Winds (SHWW) alters the magnitude of upwelling, and influence whether the Southern Ocean acts as a net sink or source of atmospheric CO_2. In recent decades the SHWW have intensified and migrated further toward Antarctica, weakening the carbon sink. Over longer time-scales, the same mechanism has been implicated to explain the increase in atmospheric CO_2 during the last deglaciation. Unfortunately, our ability to predict future changes in the SHWW is limited. Most existing knowledge of past wind-behaviour is based on archives located outside the core wind-belt, and hence spatial resolution in many areas of the Southern Ocean is poor. Consequently, more palaeo-data from inside the core wind-belt is needed to provide boundary conditions for earth system models. One such opportunity is provided by peatland ecosystems on the sub-Antarctic islands. Peatlands on exposed west-facing coasts receive oceanic base cations in concentrations proportional to the strength of the prevailing westerly winds. Reconstruction of bog-surface salinity could provide a direct proxy for past changes in wind behaviour, but methods to suitably extract the signal are lacking. In this thesis, we propose and test the idea that testate amoebae could record bog-surface salinity conditions. Using a land-sea transect on Marion Island (sub-Antarctica) we examine the ecology of these microorganisms and test their response to the contemporary conductivity gradient. We find that conductivity explained the most variance in community data, and show the potential of our novel proxy for palaeowind reconstruction in the wider sub-Antarctic region.

Chapter 1: Introduction

1.1. Research focus

Coupled climate-carbon models predict an increase in the airborne fraction of anthropogenic CO_2 emissions due to a projected decrease in the efficiency of the land and ocean sinks (Cox et al., 2000; Friedlingstein et al., 2006; IPCC, 2013). Unfortunately, uncertainty surrounding the effects of climate change on ocean circulation, and therefore the future role of the ocean-carbon feedback, remains significant (IPCC, 2013; De Vries, 2014).

The ocean sink results from imbalanced net uptake of atmospheric carbon dioxide (CO_2) at the surface of the world's oceans (via diffusion and the biological pump) relative to CO_2 outgassing by deep-water upwelling (Anderson et al., 2009; Sigman et al., 2010). It is a critical, large-magnitude component of the global carbon cycle and an important determinant of the future Earth system (Friedlingstein et al., 2006; Gruber et al., 2009). In total, the ocean carbon sink has sequestered ~50% of emissions from fossil fuel burning, and ~30% of all anthropogenic emissions including cement production and land-use change (Gruber et al., 2009). Although once overlooked (Rintoul, 2011), today the Southern Ocean is acknowledged as a substantial sink of natural and anthropogenic CO_2; it represents ca. 40% of the oceanic total sink (Orr et al., 2001), and therefore has had an important role in moderating the rate of greenhouse warming since the industrial revolution (Le Quéré et al., 2007; Tollefson, 2016).

In response to the combined effects of stratospheric ozone depletion and anthropogenic emissions of greenhouse gases, the winds that blow over the Southern Ocean have intensified and moved towards Antarctica in the past 50 years (Thompson and Solomon, 2002; Toggweiler, 2009; Thompson et al., 2011; Mayewski et al., 2013; Watson et al., 2014). This is problematic because the winds drive circulation in a way that can increase degassing of stored carbon from deep within the ocean, and ultimately determine the strength of the Southern Ocean sink (Sigman et al., 2010). It is proposed that a similar trend occurred during the last deglaciation, and that variability in the position and intensity of this wind-belt has contributed to glacial-interglacial atmospheric CO_2 variability (Toggweiler et al., 2006; Anderson et al., 2009; Sigman et al., 2010).

However, the effect of the winds on the global carbon cycle, and specifically atmospheric CO_2 has been the focus of controversy (Toggweiler et al., 2006; Menveil et al., 2008; Anderson et al., 2009, Moreno et al., 2010). A major problem in this debate is the lack of suitable palaeo-data on past wind conditions, and hence it is also a significant limitation in our understanding of past climate (Stager et al., 2012).

Palaeoenvironmental reconstructions can play a crucial role in providing boundary conditions for fully coupled climate-carbon cycle models needed to address the debate and predict future change. Therefore, more frequent and improved reconstructions of past wind conditions are required. The sub-Antarctic islands represent an ideal opportunity to develop high-quality wind reconstructions due to their location in the Southern Ocean, and many have actively accumulating peatlands which could be suitable palaeoclimate archives. The overarching aim of this project is to test the suitability of using

soil microorganism (testate amoebae, *see section 3.2*) responses to changing peatland-surface salinity conditions as a novel and direct palaeo-wind proxy needed for future research.

1.2. Project context

This project is aligned closely to a larger ongoing investigation conducted by the British Antarctic Survey supported by the 2013-2017 NERC standard grant NE/K004514/1. The investigation has a broad spatial scope, and primarily seeks to improve the spatial resolution of available Southern Hemisphere palaeowind datasets by developing novel wind proxies from all areas of the sub-Antarctic region.

Ongoing research is testing the use of diatoms for this purpose and is yielding promising results. Macrofossil analysis, proposed as a method to determine changes in coastal vegetation succession, and XRF scanning of peat cores to identify concentrations of oceanic salts are also planned to infer changes in wind conditions. Field sampling has now been completed on Macquarie, Campbell, Marion and the Cape Horn archipelago Islands. The samples kindly provided for analysis in this thesis were collected during these expeditions.

The development of a novel proxy in this study is intended to supplement the other methods available and contribute to the development of a more expansive ‘tool-kit’ for multi-proxy analysis in the future. A multi-proxy approach to analysis is preferable because it allows a more thorough picture of environmental variability to be developed, and the possibility to cross-validate individual records. The latter is crucial because the limitations associated with each proxy require robust testing before accurate conclusions can be drawn.

1.3. Research Hypotheses

The following hypotheses are proposed:

- H^1 – ‘Testate amoebae are present in the salt influenced peatlands on the west coast of sub-Antarctic Marion Island’.
- H^2 - ‘Testate amoebae assemblages found on Marion Island vary in response to the contemporary gradient of salinity (conductivity) on the peatland surface, which is induced by wind delivered oceanic salts’.
- H^3 – ‘Testate amoebae respond sufficiently to gradients of conductivity (or other salinity measures) to allow transfer functions to be developed’.

1.4. Thesis structure

This chapter has provided a brief introduction and rationale for the research hypotheses that will be addressed. Chapter 2 develops this further and is designated to introducing the scientific background of the project. This includes discussion on the crucial role of the Southern Hemisphere westerly winds within the climate system, and outlines the mechanisms of peat formation and usage of peatland ecosystems in palaeoenvironmental studies.

Chapter 3 is comprised of three separate reviews. The first presents a synthesis of published research on the sub-Antarctic peatlands, including a review of their bioclimatic space, age, accumulation rates and disturbance history. The second introduces and reviews the use of testate amoebae as

palaeoenvironmental indicators. A wide scoped synthesis of existing testate amoebae studies throughout the Antarctic region is also presented. The third section provides an extensive review of the existing methods used to reconstruct past wind conditions over the Southern Ocean. It details the interpretations of the records, and maps their distribution for the first time. Finally, the fourth section provides the rationale behind this project, and outlines the ways that it will benefit research in the future.

Chapter 4 presents a detailed assessment of the physiography and context of study site on Marion Island (sub-Antarctic) used for this thesis. Chapter 5 presents the main research output, which assesses the hypotheses and concludes the project. With the aim of standardising testate amoebae identifications throughout the sub-Antarctic, Chapter 6 presents the secondary output, a new draft taxonomy including all of the testate amoebae identified in this study.

Chapter 2: Scientific Background

2.1. Southern Hemisphere Westerly Winds and the Earth System

The Southern Annular Mode (SAM), also termed the Antarctic Oscillation, is defined by the mean sea level pressure difference between 40°S and 65°S (Turney et al., 2016a). It is the principle mode of variability in atmospheric circulation over the Southern Ocean (Marshall, 2003), which is itself delineated by the sub-tropical front – an oceanographic boundary between warm salty sub-tropical water, and cold fresher water nearer Antarctica (Fig. 1). The SAM oscillates between high and low-index polarities; the former characterised by Antarctic pressures that are lower than the mid-latitudes (Marshall, 2003). During these phases the circumpolar vortex strengthens, surface airflow - *termed the Southern Hemisphere Westerly Winds (SHWW*) - over the Southern Ocean intensify (Marshall, 2003), and the wind-belt migrates poleward.

With the exception of the Andes in South America, SHWW circulation is unhindered by topographic barriers to circumpolar rotation, and are therefore a strongly zonal and symmetric component of the climate system (Fig.1; Fletcher and Moreno, 2012). Currently, the wind belt covers ~30-60°S (Lamy et al., 2010; Fletcher and Moreno., 2011; Knudson et al., 2011) and reaches maximum intensity in the sub-Antarctic latitudes between 50-60ºS (Saunders et al., 2015) where they are the strongest time-averaged oceanic winds found globally (Russell et al., 2006; Hodgson and Sime, 2010). However, their

latitudinal position and intensity undergoes shifts at a variety of timescales. Seasonally, the wind-belt contracts poleward and strengthens during summer and in winter expands northward, and weakens (Hodgson and Sime, 2010; Lamy et al., 2010; Varma et al., 2012). Over inter-annual to decadal timescales, variability is driven by atmosphere-ocean phenomena (e.g. SAM and El Niño) which adjust the pressure systems that direct westerly airflow (Knudson et al., 2011). Over millennial (glacial-interglacial) time-scales, variability appears to be linked to changes in global climate (Toggweiler, 2009).

The observational record indicates that since the 1950s the winds have undergone a poleward shift and strengthening, associated with SAM trending to a positive high-index state (Marshall, 2003; Dixon et al., 2012). These changes have been attributed to the depletion of stratospheric ozone (Thompson and Solomon, 2002), and models indicate that rising greenhouse gas concentrations may have also played a role (Thompson et al., 2011). The poleward shift has been in the magnitude of several latitudinal degrees (~3.5-2°S for winter and summer respectively) and winds have intensified by ~6% (1981-1990 relative to 2001-2010) (Mayewski et al., 2013). Records from Antarctic ice-cores suggest that such change is unprecedented over the last 100,000 years (Mayewski et al., 2013), and is projected to persist through the 21st century with further warming (Toggweiler and Russell, 2008; Stager et al., 2012; Bracegirdle, 2013; Mayewski et al., 2015).

Circulation of the Southern Ocean is strongly forced by the SHWW. Crucially, they are associated with the Antarctic Circumpolar Current (ACC) (Chavaillaz et al., 2013) and the upwelling limb of the meridional overturning circulation. Upwelling occurs because the winds push surface waters away from the

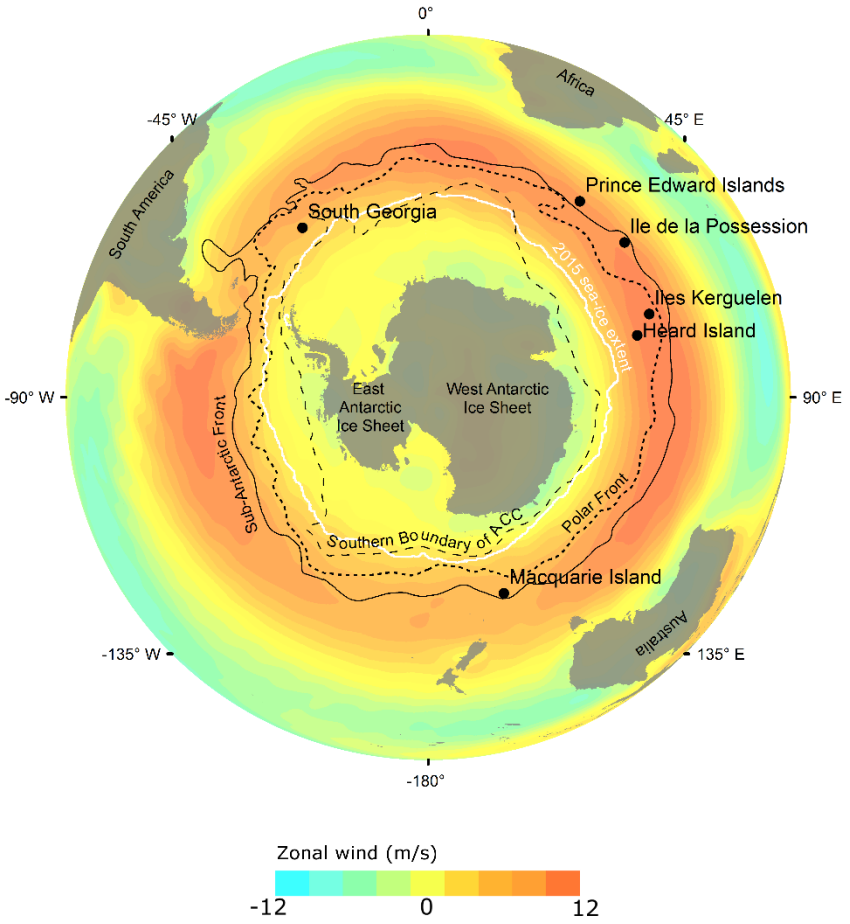

Figure 1 | Location of the larger sub-Antarctic islands which lie inside the core westerly wind belt and in relation to the position of the; Southern Boundary of the Antarctic Circumpolar Current (ACC), polar front and sub-Antarctic front. Westerly wind belt shown using 20th Century NCEP re-analysis data of 1000 mb zonal wind (composite mean) from 1982-2012 (Kalnay et al., 1996; NOAA, 2016). Positive values indicate airflow in the westerly direction and negative values indicate easterly flow. Maximum extent of sea-ice in the 2015 season is also delineated by the white line. Marion Island, used in this report, is part of the Prince Edward Islands.

Antarctic coast via Ekman transport (Russell et al., 2006) and therefore continually 'pump' dense interior ocean water to the surface (Toggweiler and Russell, 2008).

Further, recent research has invoked a hypothesised ocean-SHWW coupled system, where the influence of winds on large-scale ocean circulation are used to partly explain past atmospheric CO_2 variations (Fletcher and Moreno, 2011, 2012). However, the extent to which the system could change atmospheric CO_2 remains uncertain (Moreno et al., 2010). The system is proposed to operate when changes in SHWW behaviour, namely position and strength,

promote degassing of deep water via perturbation of upwelling in the Southern Ocean releasing carbon to the atmosphere, and vice versa. Together with ocean biogeochemistry this system influences whether the Southern Ocean acts as a net source or sink of atmospheric CO_2 (Toggweiler et al., 2006; Le Quére et al., 2007); Toggweiler and Russell, 2008; Toggweiler, 2009; Anderson et al., 2009; Hodgson and Sime, 2010; Sigman et al., 2010).

During the last deglaciation, a large quantity of oceanic CO_2 entered the atmosphere, raising the atmospheric concentration by approximately 50% (Hodgson and Sime, 2010). Toggweiler et al. (2006) suggest that this injection was driven by a southward migration of the SHWW ~7-10°S of their glacial position, that aligned them more squarely with the ACC, strongly increasing ventilation of the deep ocean and increasing degassing of 'old-carbon' at the surface (Toggweiler and Russell, 2008). Further, they demonstrate that the wind-belt tends to be shifted poleward during warm (interglacial) periods, whereas during cold (glacial) periods they tend to occupy a more equatorward position. They propose that glacial atmospheric CO_2 concentrations were supressed because reduced wind-driven upwelling allowed a more stratified ocean and therefore carbon accumulated in the deep ocean. The theory, which is based on a general circulation model, is seemingly supported, at least in direction of change, by some initial empirical data (e.g. Moreno et al., 2010; Whittaker et al., 2011), but there is active debate (Menviel et al., 2008). Anderson et al. (2009) present a record of ocean productivity through several glacial cycles that track periods of enhanced wind-driven upwelling during deglaciations, which coincided with increasing atmospheric CO_2 concentration and temperature recorded in Antarctic ice-cores. It is important to acknowledge that this study demonstrates only that key changes occurred concurrently and

does not necessarily prove causality. The observational record also lends some support by convincingly demonstrating that an increase in global temperatures has occurred concurrently with shifting and intensifying westerlies.

The Southern Ocean acts as an important sink of anthropogenic CO_2 (i.e. non-natural carbon emitted to the atmosphere since the Industrial Revolution) and therefore it has partially buffered the effects of anthropogenic climate change. Because carbon sequestration depends strongly on circulation, observations of the recent wind field intensification have been shown to reduce the efficiency of the carbon sink (Le Quéré et al, 2007).

Beyond the carbon cycle, the effects of the SHWW on the Southern Ocean are important for a variety of reasons. Antarctic Peninsula has warmed by ~0.56°C per decade since the 1950s (Turner et al., 2009), and although there has recently been a reported shift to overall cooling since the late 1990s (Steig, 2016) it is acknowledged that warming on the east coast is related to poleward migration of the westerlies (Steig, 2016). Strengthening westerlies also drive the incursion of warmer Circumpolar Deep Water against the grounding lines of the adjacent Antarctic Ice-sheets (Spence et al., 2014b), a major driver of basal melting. For each 1°C ocean temperature increase basal melt rates can increase by ~10 m yr^{-1} (Rignot and Jacobs, 2002). The reverse bed-slopes of the West Antarctic Ice Sheet make it inherently vulnerable to runaway retreat under scenarios of enhanced warmer water ingress at the grounding line, which could lead to rapid collapse (Mayewski et al., 2015). For example, modelling suggests that stronger westerly winds over the Amundsen sea were responsible for more rapid basal melt of surrounding ice-shelves, followed by

a reduction in latitudinal stress and consequential acceleration of glacier flow (Bracegirdle, 2013). The winds may also alter sea ice extent and thickness (Bintanja et al., 2013) and affect the rate of deep-water formation (Spence et al., 2014a). Sea-ice is important for maintaining the Earth's albedo and regulating air-sea greenhouse gas fluxes.

The SHWW dominate the climate and influence precipitation patterns between 30-70°S (Fletcher and Moreno, 2011; Varma et al., 2012). They influence the amount and distribution of precipitation in the southern continents (Moy et al., 2008), and therefore accurate predictions of how they will change in the future are important for the provision of water and agriculture in this region.

Currently, the SHWW are relatively poorly represented in models with most attempts simulating the belt of maximum intensity far equatorward of the observed position (Russell et al., 2006), especially when compared to palaeodata. Additionally there are few records of wind-behaviour during more recent short climate intervals (e.g. Little Ice Age, and Medieval Climate Anomaly). These periods may be important in generating more informed conclusions (Moy et al., 2008), and understanding if the SHWW-Southern Ocean circulation-atmospheric CO_2 system constitutes a positive climate feedback, by testing their response to climate warming and cooling.

For the reasons outlined in this section, it is becoming increasingly important to contextualise the recent intensification and migrations of the SHWW with analogous periods in the past, and better model how they will continue perturbing the earth system in the future. To achieve this more reliable palaeowind reconstructions from the Southern Ocean region are needed.

2.2. Peat and Peatlands

Peat landforms (*'peatlands'*) refer to biotic landscapes formed of peat; a sedentarily accumulated carbon rich soil comprised of partially decayed remnants of plants that once grew at the surface (Clymo et al., 1998). As ecosystems, peatlands are characteristically waterlogged, unproductive, low in nutrients, and often acidic, with plant and microbial communities adapted for these conditions. Anoxia of the soil matrix and acidity supress decomposition so that carbon inputs from plant productivity exceed decay, facilitating vertical accumulation of peat over time.

Active accumulation requires a positive moisture balance (i.e. low temperature and/or high precipitation), and therefore climate conditions strongly influence the peatland distribution. Suitable bioclimatic regions occur worldwide, and allow peatlands to occupy ~3% of the global land area (Yu et al., 2010). Nomenclature of peatland types varies greatly between regions and languages, but they can be categorised at the coarsest scale as either tropical (30°N-S of the equator) or high-latitude (temperate and boreal regions).

The importance of peatlands; (1) in the global carbon cycle, (2) for their biodiversity and (3) as archives of past environments, attracts substantial research interest in them. In total they store 600 Gt of carbon sequestered from the atmosphere since the Last Glacial Maximum (Yu et al., 2010; Charman et al., 2013). Northern high latitude peatlands hold a disproportionate 547 Gt C ($4x10^6$ km^2) and tropical regions contain ~50 Gt C (368,500 km^2) (Yu et al., 2010). Due to limited available land-areas, Southern high-latitude peatlands contain just ~15 Gt C (45,000 km^2), and are mainly located in Patagonia and

New Zealand (Yu et al., 2010). Limited peat deposits are also present in both the continental- and sub-Antarctic regions, and these rare, understudied environments are the subject of this project.

All accumulating peatlands grow vertically with each productivity year, as successive growth layers are added to preserved layers beneath. Each layer incorporates information of biotic and abiotic conditions at the time that it formed, which can be recovered and used to obtain palaeoclimate information. Peatlands are especially useful in palaeoclimate studies since they accumulate organic matter at a relatively fast rate and this produces a record of high temporal resolution.

Use of peatlands in palaeoclimatology can be separated into two major categories. First, the initiation of peatland environments can be used to constrain dates of events such as deglaciation (Hodgson et al., 2014) or to identify broad climatic shifts to wetter and/or colder conditions from periods of large scale peatland expansion (Gallego-Sala et al., 2016). Second, the constituents and properties of the peat itself can be used to gain higher resolution information on environmental changes through the period of peat development (Charman, 2002). For this usage, peatlands can be seen as; (1) records of autogenic change (i.e. changes within the peatland ecosystem itself), and (2) preservers of materials indicative of allogenic changes in the wider non-peatland environment (i.e. as depositories of allochthonous material) (Charman, 2002).

A variety of threats including direct anthropogenic pressures (e.g. drainage, pollution and extraction) and climate change (e.g. temperature increase, precipitation decline) endanger continued persistence of peatlands.

Palaeoenvironmental reconstructions require continual accumulation and long-term preservation of the record and therefore many archives are (and increasingly will be in the future) rendered unusable because of anthropogenic disturbance.

2.2.1 Peat accumulation and peatland development

Development of the peat record relies on a maintained positive carbon balance, i.e. atmospheric carbon drawdown must exceed the losses via numerous export pathways. A positive carbon balance relies on total decomposition in the peat matrix being lower than primary productivity on the peatland surface. The rate of productivity responds to variability in: nutrient and water availability, temperature, atmospheric CO_2 concentration and photosynthetic active radiation (Loisel et al., 2012). Similar factors (oxygen content, pH, temperature, organic matter properties, and nutrient availability) control the rate of decomposition, and how sensitive these two processes are to different environmental change will determine the overall rate of peat accumulation.

Individual processes act over two functional layers in the vertical cross-section of the peatland; the surface acrotelm, delineated by the minimum water-table position and the underlying catotelm that extends to the substrate beneath the peat deposit (Ingram, 1978).

Within the acrotelm photosynthesising plant tissue in the euphotic zone draws CO_2 from the atmosphere, and this comprises the most significant origin of peatland carbon inputs. The balance between carbon gained via photosynthesis and lost by plant and peat-matrix respiration is termed the net ecosystem exchange and is positive in an accumulating peatland. The

acrotelm is aerated, not permanently below the water table, and consequently ~90% of biomass inputs are lost by respiration and aerobic decomposition (Rydin and Jeglum, 2013). Organic matter, which avoids decay is gradually transferred to the waterlogged (anoxic) catotelm beneath, as the peat sequence develops above.

The catotelm is characterised by a shift to anaerobic decomposition and to more recalcitrant material so rates of decay decline sharply relative to the acrotelm. Peat bulk density increases beyond this transition due to compression by successive growth layers above. Gradually, the pressure causes structural collapse within the peat impairing hydraulic conductivity which facilitates a permanent high-water table (Belyea and Malmer, 2004). Although anoxia supresses decomposition to allow millennial time-scale storage of organic matter it does not reduce to zero. Methanogen-bacteria inhabiting the anoxic zone digest organic matter to produce methane that is transferred to the surface via bubbling, plant aerenchyma and diffusion. Consequently peatland development is finite, and long-term accumulation rates will gradually reduce as the ecosystem develops (i.e. once the sum total of decomposition in the peat sequence equals production at the surface accumulation will stop) (Clymo et al., 1984). This is the limit on the time-period that it is possible for continuous peat records to span.

2.2.2 Peatland types and their palaeoenvironmental uses

There are two major classifications of peatlands, which principally concern nutrient status, and affect the way they can be used in palaeoenvironmental studies. Ombrotrophic bogs, receive all of their water solely from precipitation falling directly onto the peatland surface. They are typically highly acidic and

have very low nutrient concentrations. As they develop the bog-surface height increases, growing away from the water table and forming a domed shape. Due to their isolation from ground water sources and reliance on atmospheric moisture delivery, ombrotrophic peatlands are frequently used as archives of past precipitation conditions. Palaeoenvironmental methods that are used to achieve this include testate amoebae transfer functions and isotopic oxygen analysis. They are also seen as preferable preservers of allochthonous material (e.g. Orme et al., 2015), since material must be directly deposited on their surfaces and not brought in by run-off from elsewhere.

Minerotrophic fens are influenced by water from outside the boundary of the peatland itself (i.e. groundwater and run-off) as well as precipitation. They are typically richer in nutrient, and have a higher pH. As most peatland types initiate as fens, they can be used successfully in studies that consider the timing of peat development. However, the link between surface wetness and climate is weaker than in ombrotrophic bogs (Payne, 2011), and therefore they are considered less appropriate archives for precipitation reconstruction. Additionally, since material deposited in a wider catchment can enter fens, they are considered sub-optimal for studies of atmospheric allochthonous particle deposition.

Chapter 3: Literature Review

3.1. Peatlands in the sub-Antarctic region

3.1.1. Current research

Southern Hemisphere peat deposits occur abundantly in Patagonia, Tasmania and New Zealand (Yu et al., 2010; Rydin and Jeglum, 2013). Further South is the sub-Antarctic, a section of the Antarctic biome located between the polar and sub-tropical front in the Southern Ocean. Although the region has no continental landmasses, six major island groups are present (Fig. 2), and have a combined land area of ~26,000 km^2 (Hodgson et al., 2014). All have bioclimatic space suitable for peat development (Table. 1; Wieder and Vitt, 2006), and deposits have been documented on each island (Table 2). However, more quantitative records are lacking; current bioclimatic models (e.g. Gallego-Sala and Prentice, 2012) are not suitably resolved to identify them, and they are poorly represented in current inventory-based mapping (e.g. Yu et al. 2010).

Extreme isolation and limited ice-free land-areas within the Southern Ocean make these peatlands rare and highly distinctive at the global scale. From existing literature descriptions they do not appear to conform ecologically or morphologically to many of the general principles established in boreal peatland science. Further examination is needed to determine whether there are analogues for these peatlands in climatically similar high Northern latitudes, for example if they share similarities with blanket bogs or permafrost peatlands.

Table 1 | Bioclimatic conditions of the six major sub-Antarctic Island groups with records of accumulating peatlands. MAT; mean annual temperature, CMA; coldest month average temperature, WMA; warmest month average temperature. Seasonal temperature variation, calculated as the difference between warmest and coldest months. Note the high degree of variability in temperature and precipitation values for each location, which is attributed to the rapidly changing conditions and differences between weather stations due to orography. * Information on current permafrost presented by Boelhouwers et al. (2003) *and references within*.

Island	Nearest weather station reference (GHCND)	Island Size (km^2)	MAT (°C)	CMA (°C)	WMA (°C)	Seasonal temperature Variation (°C)	Direct sun hours/day	Annual precipitation (mm)	Current permafrost*
Prince Edward Island	No Station: Nearest is Marion Island.	90	5.6-6	4.0	8.0	4.0	3.7	2000-2400	Sporadic above 1,000 m above sea level (a.s.l)
Marion Island	SF000068994	290							
île de la Possession	FSM00061997	146	5.0-5.5	2.9-3.0	7.9-8.0	5.0		2200-2400.	Uncertain
îles Kerguelen	FRM00061998	7200	4.6-5.5	2.0	9.0	7.0		700-3200	Potentially at high altitude
Heard Island group	No Station: Nearest is Iles Kerguelen	368	2.0	0.0	4.0	4.0-4.5			Yes
Macquarie Island	No Station: Nearest is Campbell Island	123	4.8-5.0	3.0-3.3	7.0	4.0	2.2	895-900	Not present
South Georgia	SG: SXM00088903 Bird Island: SXM00088900	3900	1.5	-2 - -1.2	5.0-5.6	7.0		1600	Potentially at high altitude

The low-diversity of plant species found on the islands make the peatlands unusual floristically, in contrast with the rich biota found in Northern peatland areas. Also few of the insects, amphibians, mammals or birds that are characteristic of equivalent latitude boreal peatlands are present (Rydin and Jeglum, 2013). These differences affect ecosystem functioning, for example there are no macroherbivores on Marion island, so loss of primary production by grazing is negligible (Smith, 2002). Many of the peatlands do not have a surface patterning (mound and hollow), and *Sphagnum* species, a key peat builder (van Breemen, 1995) with global distribution are either absent or of negligible importance to peat formation (Van der Putten et al., 2012a).

The literature suggests that sub-Antarctic peatlands are impacted less by anthropogenic threats such as pollution or drainage than other peat abundant regions worldwide. The islands have only been exposed to sporadic visitation and transitory settlement since the late Eighteenth Century. However, early exploration, primarily driven by the sealing, whaling and fishing industries, caused extensive damage to both marine and terrestrial ecosystems, especially on Îles Kerguelen where much of the native flora was destroyed (Campos et al., 2013). Today, robust protection measures are in place; all islands except the Heard group are inhabited for scientific research purposes and tourism is a currently restricted but growing industry (Campos et al., 2013). It is apparent that anthropogenic perturbations of the sub-Antarctic peatlands are primarily via impacts to their flora, however early visitors also introduced several species of non-native vertebrate grazers and insects. Climate change is an additional emergent threat to peatland ecology and functioning.

Except for moss banks on the Antarctic Peninsula, which are outside the sub-Antarctic, all of the regions peatlands are categorised as oligotrophic to eutrophic fens since they receive water from beyond their own limits (Van der Putten et al., 2012a; Charman, 2002). Enrichment by oceanic base cations is an important feature of sub-Antarctic peatlands and other vegetation communities. Base cations are deposited by two major mechanisms; (1) High concentration input from waves generating direct splashes or inundation, especially during storms, and (2) Low concentration input from airborne sea-spray incorporated into the wind and deposited via precipitation (Bergstrom and Selkirk, 2000).

Land-area in the sub-Antarctic is limited (Fig. 2) and so the peatlands there are extremely valuable terrestrial archives of palaeoenvironmental information (Van der Putten, 2012a). As with peatlands worldwide they are stratified deposits, with annual growth layers that could preserve long-term, accessible, and high-resolution records of their own development including the deposition of foreign materials on their surfaces (Yeloff et al., 2007). In the last decade, they have been used for a variety of palaeoenvironmental investigations (e.g. Yeloff et al., 2007; Van der Putten et al., 2008; McGlone et al., 2010; Van der Putten et al., 2015). Their usage in palaeoenvironmental reconstruction has been the subject of a review by Van der Putten et al. (2012), however the scope of this study was largely restricted to a description of the peat-forming vegetation and presentation of newly obtained conventional palaeoclimate data. Therefore, a wider reaching review including information on rates of accumulation, timing of initiation, the mechanisms of peat development, nutrient status, and details about preservation for key proxies is urgently required.

Table 2 | Descriptions and accumulation rates of peat forming ecosystems on the six major sub-Antarctic islands inside the core westerly wind belt. Radiocarbon dates at the lowest sampled point within peat cores (basal dates) were selected for inclusion. Average accumulation (accretion) rate over the period of peat growth was calculated by dividing depth by age.

Vegetation and Peatland Notes	Peat Depth, Mean date *2-sigma range* (k yr B.P.)	Source	Average peat depth ± S.E (cm)	Standard deviation	Age ± S.E (k yr B.P.)	Standard deviation	Average accretion rate ± S.E (mm yr^{-1})	Standard deviation
Prince Edward Islands (Marion)								
• Peatlands cover ~50% of the island below 300 m a.s.l., and do not possess a surface patterning of mounds and hollows, although open-water pools are present. • Nutrient status characteristically oligotrophic to minerotrophic (Van de Vijver et al., 2008). • Peat forming plant communities are dominated by graminoid spp. (Van de Vijver et al., 2008; Yeloff et al., 2007) notably *Agrostis magellanica, Uncinia compacta, Juncus scheuchzeriodes* (Yeloff et al., 2007). • Peatlands also contain bryophytes including *Racomitrium lanuginosum.* • Locally very deep peat deposits occur up to 9 m, and it is suggested that some may pre-date deglaciation.	261.5 cm, **5.50** 600 cm, **8.07** (*7.93-8.20*) 130-140 cm, **7.72** (*7.57-7.87*) 353-363 cm, **6.78** (*6.60-6.95*) 165-180 cm, **4.59** (*4.43-4.74*) *300 cm, **17.32** (Extrapolated)*	Yeloff et al. (2007) Scott (1985) Scott (1985) Scott (1985) Scott (1985) Van der Putten et al. (2010)	305.4 ± 3.12 *304.5 ± 7.87*	185.864 *166.256*	**6.53** ± 0.66 ***8.33** ± 1.88*	1.473 *4.597*	0.460 ± 0.09 *0.412 ± 0.09*	0.208 *0.220*
Île de la Possession								
• Mosses form a major component of vegetation and peat coverage is extensive at low altitudes (Van der Putten et al., 2008). • Pre-Holocene peat deposits are unlikely due to the influence of erosion and mid-Holocene volcanism (Van der Putten et al., 2010). Peat initiation dates most likely represent a volcanic event rather than deglaciation.	532 cm, **6.16** (*6.00-6.32*) 402 cm, **10.88** (*10.75-11.00*) 197 cm, **7.02** (*6.78-7.02*)	Van der Putten et al. (2008) Van der Putten et al. (2010) Ooms et al. (2011b)	337.00 ± 97.51	168.893	**8.00** ± 1.46	2.536	0.507 ± 0.18	0.319
Îles Kerguelen								
• Mire communities are present but generally restricted to valley floors and/or areas of impeded drainage (Van der Putten et al., 2015). • Peat deposits >5 m have been recorded.	468 cm, **16.01** (*15.39-16.62*) 525 cm, **13.00** (*12.77-13.24*) 260 cm, **9.53** (*9.14-9.91*) 206 cm, **13.89** (*14.04-13.73*)	Van der Putten et al. (2015) Young and Schofield (1973) Young and Schofield (1973) Henao (2015)	364.75 ± 77.74	155.472	**13.11**±1.35	2.699	0.279 ± 0.05	0.105

Heard Island group								
• Peat deposits up to 2 m deep, mainly comprised of *Azorella selago* have been reported. Coalesced *Azorella selago* cited as most important peat forming vegetation (Bergstrom et al., 2002). • Deposits are isolated to low-altitude areas where primary production is relatively high. These areas are mainly Sydney Cove, Long Beach and Dovers moraine, where depths exceeding 1 m are recorded (Bergstrom and Selkirk, 2000). • The island is mostly covered by ice and only ~5% has vegetation cover (Bergstrom and Selkirk, 2000).	Dovers moraine underlying a 2 m peat sequence is expected to date from the Holocene (Hodgson et al., 2014). **No radiocarbon dating**, or information on glacial history since the LGM.							
Macquarie Island								
• The island has extensive peatlands. Peat forming vegetation is dominated by *Juncus scheuchzerioides.* Bryophytes include *Sphagnum falcatulum.* • Lowland areas covered by up to 6 m deep peat deposits. Many are located on a raised coastal terrace, where vegetation is dominated by mosses and lichens. Quaking mires are located a Handspike Point and Half Moon Bay, and have a patterned surface (Macquarie Island Management Plan, 2006). • The island is prone to earthquakes that trigger land-slips in the peat deposits, which may mean that records are not continuous.	190 cm, **6.74** (*6.21-7.27*) 130 cm, **7.94** (*7.68-8.20*) 182 cm, **11.93** (*11.28-12.58*) 360 cm, **5.89** (*5.59-6.20*)	Selkirk et al. (1988) Selkirk et al. (1988) Selkirk et al. (1988) Selkirk et al. (1982)	215.5 ± 49.97	99.938	**8.13** ± 1.34	2.672	0.302 ± 0.11	0.214
South Georgia								
• Ice-cover is extensive and reaches sea level. • Areas free from ice are covered with tundra-like vegetation. Peat cover is locally expansive and developed at lower altitudes. • Peat depths are of variable thickness, 1-5 m. • Moss peat banks are present, formed of *Polytrichum strictum* and *Chorisodontium aciphyllum*. Typically 1-2 m deep, and occasionally 3 m (Van der Putten et al., 2009). • Peat deposits are also reported at 'Top Meadows' on Bird Island immediately to the North.	373 cm, **8.92** (8.72-9.12) 308 cm, **10.70** (10.51-10.89) 300 cm, **7.63** (7.57-7.69) 258 cm, **10.90** (9.65-12.15) 350 cm, **11.08** (10.55-11.60) 460 cm, **10.34** (10.11-10.57) 160 cm, **9.48** (9.40-9.55) 312 cm, **9.14** (9.00-9.27) 290 cm, **7.30** (7.17-7.42) 460 cm, **9.67** (9.54-9.80) 180 cm, **9.59** (9.50-9.68)	Hodgson (Unpublished data) Van der Putten et al. (2004) Van der Putten et al. (2013) Barrow (1978) Van der Putten and Verbruggen (2005) Van der Putten et al. (2012a) Barrow (1978) Van der Putten et al. (2009) Van der Putten et al. (2013) Smith (1981) Smith (1981)	313.73 ± 29.08	96.451	**9.52** ± 0.37	1.242	0.333 ± 0.03	0.104

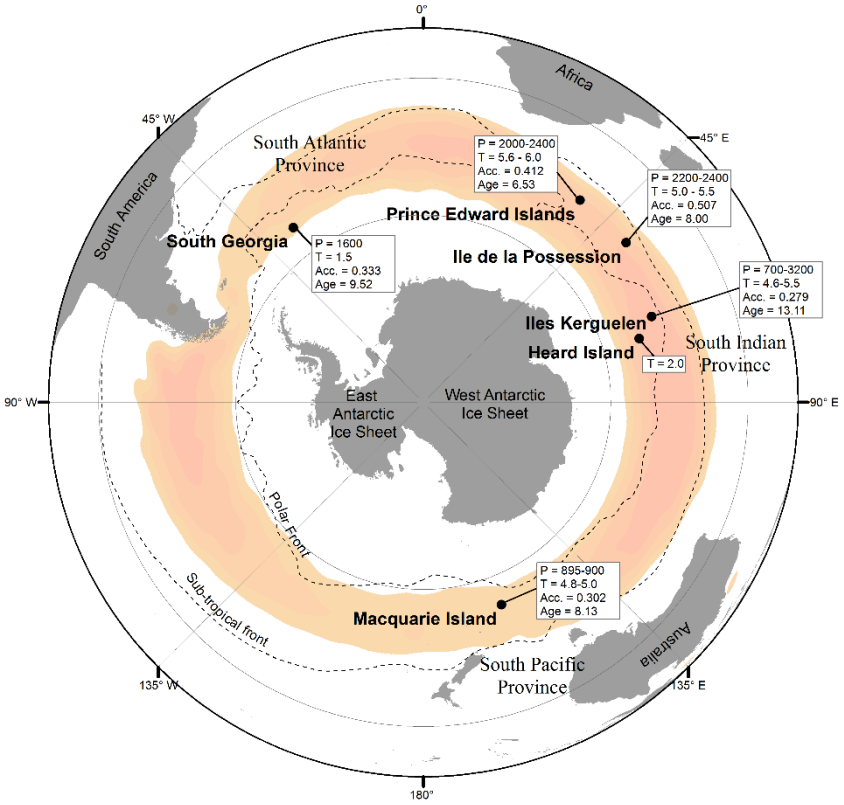

Figure 2 | Map of the sub-Antarctic island groups and provinces, including South Georgia in the south Atlantic, the Prince Edward Islands, Îles de la Possession (Crozet), Îles Kerguelen, and Heard Island in the south Indian Ocean, and Macquarie Island in the South Pacific (Van der Putten et al., 2010). Boxes provide yearly average precipitation values (P) and mean yearly temperature (T). Accumulation rate (acc.) of peatlands deduced from studies publishing radiocarbon dates (*see* Table 2) are shown in mm yr^{-1} and the average age of peat cores obtained from each island (Age). The band of most intense westerly winds (from NCEP reanalysis data (Kalnay et al., 1996; NOAA, 2016) presented in Fig. 1, demonstrates the position of the sub-Antarctic islands within the wind-belt). Temperature on South Georgia and Heard Island are significantly less than the other islands due to their more poleward latitude.

Currently, there are few studies focussing on peat initiation, subsequent growth or predictions for future persistence of peatlands in the sub-Antarctic. The former are important issues facing their utilisation as sources of palaeoenvironmental information since they determine the length (time-period) of the records that can be obtained and indicate if it is likely to be continuous. For example, working on the Prince Edward Islands, Yeloff et al. (2007) present evidence from a peat record suggesting that deposition of ash, from the islands volcano throughout the

Holocene, continually reset ecological succession and affected the peat accumulation rate.

To begin addressing the question of timing in sub-Antarctic peat initiation, studies publishing radiocarbon basal dates were assembled (Table. 2). A large number of these basal dates were recently assembled by Hodgson et al. (2014) who used them to infer the timing of deglaciation in the sub-Antarctic, which the authors assume is the primary control on peat development. It remains uncertain how long after land-areas became ice-free that peat initiation occurred, and through what mechanisms peat spread to its current extent. Other factors, such as sea-level change and volcanism, may have also affected peat formation. There is also some evidence that peatlands developed on nunatacks (glacial refugia), and hence could possibly pre-date deglaciation (Van der Putten et al. 2010). Using the basal dates with records of peat depth, average accumulation rates were calculated (Table. 2). This showed relatively slow rates of accretion (regional average, 0.376 mm yr^{-1}) a likely product of the limited growing season, and therefore this prevents analysis at the sub-decadal scale. To put this value into context, measurements from Patagonia by van Bellen et al. (2015) far exceed this value (average 1.250 mm yr^{-1}). Variability in the age-depth relationship is assumed to reflect local scale factors such as the type of peatlands sampled.

3.1.2. Future research directions

To summarise the current state of knowledge of sub-Antarctic peatlands it is possible to identify several key themes that provide future research opportunities:

1. Mapping of peatlands in the entire sub-Antarctic region.

   Areal extent of the regions peat cover is unquantified, and such a dataset would be important for establishing baseline conditions to aid future

management plans and for site selection in palaeoenvironmental research. It is possible that land-surface cover classification using high-resolution satellite imagery and targeted ground-truthing, may provide an efficient method for mapping peatland cover throughout the sub-Antarctic. At a broader scale, bioclimatic modelling may be applicable, but the paucity of observations (i.e. temperature, cloud cover, precipitation) and palaeoclimate data from the region will hinder this technique. This method may be particularly useful in predicting future changes to the regions peatlands.

2. Understanding of the peatland response to environmental change.

   The sub-Antarctic islands are experiencing rapid changes to their climate (i.e. increasing temperature, decreasing precipitation). Considering the threat of these climatic changes for other peatlands globally, and the pristine nature of sub-Antarctic peatlands, they may be useful early examples of future changes elsewhere.

3. Constraining the timing of initiation and developing understanding of mechanisms for peat growth and expansion.

   Peat initiation is a useful constraint for deglaciation, and linking peat expansion with the regions climate is a potentially useful contribution to global-scale data assemblages (e.g. Charman et al, 2013). Further data would also be helpful in determining whether peat development is controlled by the same factors throughout the sub-Antarctic.

3.2. Testate Amoebae

3.2.1. Introduction

Testate Amoebae (Protozoa: Rhizopoda) refer to a polyphyletic group that includes all unicellular, non-marine, shelled protists. Relative to other protozoan groups (e.g. Foraminifera), they have a complex taxonomy and are therefore referred to by numerous terms including: rhizopods (Grospietsch, 1971), testaceans, thecamoebians (Neville et al, 2010) and arcellacens.

Each individual comprises of a single eukaryotic cell surrounded by a resistant shell (test) that gives protection from desiccation (Charman et al., 2000; Charman, 2001; Mieczan and Adamczuk, 2015). Test locomotion, attachment and feeding, is achieved by the protrusion of cytoplasm which surrounds the nuclei of the cell (termed a pseudopodium) through an aperture in the test wall (Charman et al., 2000; Charman, 2001). They consume bacteria, fungi and other testate amoebae in soils, and contribute to nutrient cycling (Vincke et al., 2004a).

Pseudopodia structure determines the most basic phylogenetic division of testate amoebae into the classes lobosea (rounded) and filosea (pointed/filiform) (Swindles and Roe, 2007). Tests are either proteinaceous, siliceous or calcareous, and are classified by their construction (Charman et al, 2000). Idiosomic tests comprise material secreted by the parent during reproduction, and are generally smooth and regularly shaped, whereas xenosomic tests are formed of agglutinated extraneous materials (e.g. mineral grains, fungal hyphae, pollen and diatom frustules (Charman, 2001; Mieczan and Adamczuk, 2015). Intermediate forms that are neither truly xerosomic or idiosomic also exist (e.g. *Nebela* spp.). Beyond shell structure and shape, size is also used as a basis for division. Tests typically range from 20-200 µm and a high degree of intraspecific

variability is common. For example, tests of the species *Apodera vas* vary in length between 90-210 µm (Smith and Wilkinson, 2007).

Currently, simple morphological variability allows identification of ca. 2000 species (Mieczan and Adamczuk, 2015). However, the relative scarcity of studies from (less-accessible) Antarctic and tropical regions, combined with the limited number of genetic studies (Epstein and López-García, 2008) mean that documented diversity is probably a significant underestimation. The sub-Antarctic may be an important region for undiscovered microbial diversity, because many areas remain unstudied and the extreme isolation provides a high chance of endemism.

Testate amoebae occur worldwide in almost all terrestrial and freshwater biomes, extending from the tropics (Swindles et al., 2014; Reczuga et al., 2015) to both polar regions (*North:* Swindles et al. (2015a, 2015b) *and South*: Royles et al. (2013, 2016)). Their known habitat tolerance has also been expanded with the recent discovery of living specimens in thin layers of surface water on a temperate glacier in South America (Santibaneza et al. 2011), indicating their tolerance to extreme cold climate and nutrient poor conditions. More common freshwater environments which they colonise include; lakes, water courses (Vincke et al., 2004b; Heger et al. 2009), peatlands and other non-ombrotrophic wetlands (Payne, 2011).

Testate amoebae constitute up to 30% of the microbial biomass in peatlands (Swindles et al., 2015a), and are the dominant group of heterotrophic organisms found in them (Mitchell et al., 2003; Jassey et al., 2013). Their abundance typically ranges from hundreds to tens of thousands of tests per 1 cm^3 of sample (Bobrov et al., 2004).

Moderately salt influenced soils also support testate amoebae communities (Charman et al., 1998; Gehrels et al., 2001; Charman, 2001, 2002; Charman et al, 2002; Patterson and Kumar, 2002; Charman et al., 2010; Barnett et al., 2013, 2016). Suitable conditions for colonisation typically occur in higher saltmarshes, where oceanic inundation is infrequent. Frequently inundated areas, where input of salt is high, coincide with a rapid decrease in test concentrations indicating limited tolerance to salinity (Barnett et al., 2016). Although not implicitly investigated, these studies imply that testate amoebae community structure is sensitive to control by salinity.

The following section introduces the application of testate amoebae as useful palaeoenvironmental indicators, and their specific use from peatland archives. It then examines some of the general limitations of the method that are considerations for this project. The final section presents a newly compiled and wide-scoped review of testate amoebae studies from the sub-Antarctic and Southern Ocean region. The aim of this compilation was to assess the current state of testate amoebae research in the Antarctic, to identify trends in diversity, and highlight key areas that would benefit from further research.

3.2.2. The role of testate amoebae in palaeoenvironmental studies

Testate Amoebae are well established palaeoenvironmental indicators which have been studied continuously for over a hundred years (Tolonen, 1986). They are useful because each taxon has a specifically defined ecological optima and tolerance, and responds differently to perturbations of the local environment. The overall species composition and abundance of testate amoebae in a sample therefore records a unique signature of the dominant environmental conditions at a specific location. The environmental controls, which govern community

structure can therefore be reconstructed. These include; water table depth, soil moisture content, pH, conductivity, concentration of nutrients (N, P, K, Ca, Mg), temperature, organic matter content and position in the tidal frame.

Living testate amoebae, in peatlands, inhabit the euphotic zone; a narrow layer at the acrotelm surface. As organic material accumulates at the surface with each successive growth season, the initial acrotelm is compressed and empty tests are incorporated into the bulk peat. Eventually the material is transferred to the catotelm, below the water table, where conditions facilitate long-term preservation. Compression reduces the impact of vertical zonation by living tests that inhabit sub-surface layers, and a contemporaneous record is produced (Fig. 3).

When the cell dies, its cytoplasm and nucleus decay but the resistant test remains intact for long periods. In addition to peatlands, other commonly used archives for testate amoebae analysis include: lakes (e.g. Beyens et al., 1995), and salt marshes (e.g. Charman et al., 1998; Barnett et al., 2016).

Assuming suitable conditions testate amoebae can reproduce 10-27 times per year (Charman, 2001). Conditions affecting reproduction depend on factors including; temperature, light, oxygen and food availability (Mieczan and Adamczuk, 2015). They therefore offer a substantial benefit compared to other biological proxies by providing a high resolution record that is reactive to short term and relatively low amplitude environmental changes. An additional advantage is that testate amoebae are bonded permanently to their depositional substrate, and are destroyed if the sediment is reorganised. Hence, except for potential distortion of the peat sequence by plant roots, testate amoebae are

immobile and therefore directly related to environmental conditions at the time of sediment formation (Bobrov et al., 2004).

Statistical transfer functions allow the environmental variables that control species assemblages to be quantitatively reconstructed from counts of fossil testate amoebae in a sediment core. Development of a transfer function requires a training-set of modern species responses to analogous environmental variability at the present surface. For example, on peatlands the mound to hollow gradient is commonly used to determine how living testate amoebae respond to variation in water table depth and moisture content. The training set can then be compared to fossil assemblages to provide an environmental reconstruction for conditions at the time of formation.

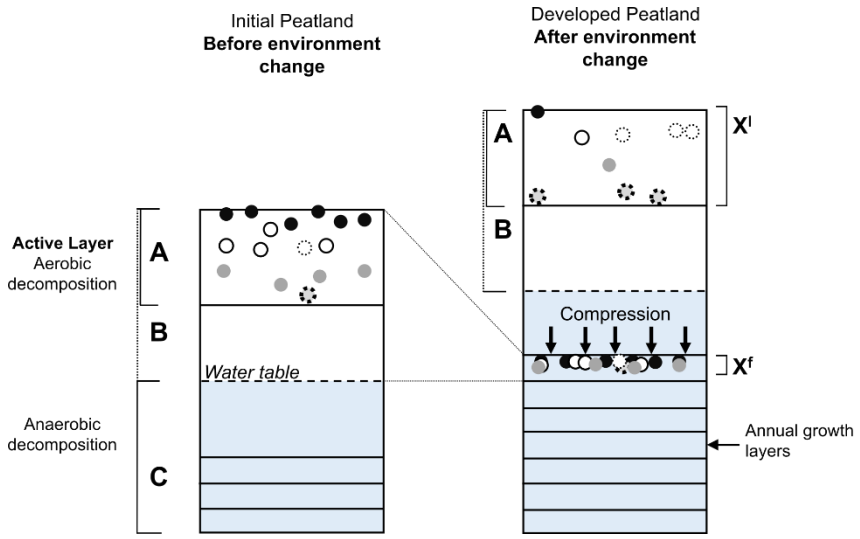

Figure 3 | Development of the testate amoebae record in an accumulating peatland. A, represents the euphotic layer which is the site of primary production and living testate amoebae, B, delineates the acrotelm. C, shows the catotelm, permanently below the water table, and the site of anaerobic decomposition. X^l vertical zonation of living tests at the surface, X^f vertical zonation of fossil tests in the catotelm after compression. Note that testate amoebae species assemblage on the peatland surface changes due to environmental perturbation between the time-step from initial to developed stage (left and right diagrams).

A separate usage of testate amoebae in palaeoenvironmental studies is aiding the retrieval of cryptotephra (i.e. tephra <125 µm) that is increasingly used to develop chronologies for palaeoe-records (Delaine et al., 2016). Due to its small size cryptotephra can be found much further from the source, allowing more dating features per archive, however this also makes it hard and time-consuming to isolate from bulk material. Since testate amoebae have been shown to incorporate mineral particles from the immediate environment into their shells, they essentially concentrate these particles. Simple sieving to extract the testate amoebae speeds-up the laboratory analysis and efficient investigation of cryptotephra.

3.2.3. Testate Amoebae based palaeoclimate studies from peatlands

Testate amoebae community restructuring in response to water table changes in ombrotrophic peatlands, provides a proxy for changing precipitation conditions (e.g. Charman et al., 2001; Swindles et al., 2014; van Bellen et al., 2015; Amesbury et al., 2016). These studies first establish the relationship of hydrology and testate amoebae via multivariate ordination analysis, before applying a training set of modern testate amoebae and hydrological conditions for reconstruction based on fossil assemblages. These studies require ombrotrophic peatlands where water table depth is controlled by precipitation only. Increasingly these studies focus on a wide geographical area and incorporate a large number of samples and observation.

Using the same concept, they are also used for environmental monitoring, for example the response of ecosystems to restoration or early detection of damage (Patterson and Kumar, 2002). Although moisture conditions strongly control testate amoebae species assemblages there are many other factors that

contribute. However, these relationships are much less intensively investigated. Recently, testate amoebae have been proven as accurate indicators for sea level reconstruction (*see* Barnett et al., 2016), and have also been used as a proxy to track variations in total microbial activity through time (Royles et al., 2013).

3.2.4. Testate amoebae analysis: advantages and sources of uncertainty

Methodologically, testate amoebae analysis is advantageous compared to many other palaeoecological techniques. Field sampling is straightforward, and requires only basic peat coring equipment. Laboratory preparation does not require the use of hazardous substances, such as the Hydrofluoric acid needed for palynology. Sample processing is efficient, involving disaggregation of tests from the organic material and concentrating them by removal of unwanted particles via sieving. Species of fossil and living tests are relatively easy to identify using a light-microscope and therefore data-collection can be completed relatively quickly.

Notable limitations and sources of uncertainty associated with the technique also exist. At small spatial scales, assemblages vary along multiple micro-environmental gradients on the peatland surface and this is superimposed onto the gradient of the desired variable. Therefore, it is difficult to precisely isolate signals from individual variables, especially when only a limited number of samples is available. Further uncertainty arises due to potential synergistic or antagonistic species and environment interactions, and when environmental variables are correlated together.

As described above, living testate amoebae colonise both the peatland surface and near subsurface, so it can be difficult to accurately define what represents a true ‘surface’ sample for training-set development. When quantifying down-core

assemblages this limitation is often overstepped by assuming that because peat bulk density increases with depth, the layer comprising the initial acrotelm is gradually compressed to produce a (thinner) contemporary record of fossil assemblages (Fig. 3). Bulk density is therefore an important measure, and may vary through the core due to environmental changes (such as decomposition or production rate, or trampling by animals).

Another issue concerns field sampling when there is a lack of suitable modern environmental analogues to establish accurate testate amoebae-environment relationships. If suitable analogues are not available, then the entire gradient of possible environmental variability cannot be encapsulated and this introduces uncertainty into the resultant reconstruction (i.e. the extremes of possible environmental gradients are omitted making it impossible to infer all possible changes). When samples are taken from small-localised sites there may be poor or even no available analogues for some species present in the fossil record (Charman et al., 2007). Variability in the fossil assemblages, which are unknown at the time of sampling, are often greater than modern variability at the same site and therefore modern analogues are unavailable. Uneven distributions of samples along the environmental gradient can also reduce transfer function performance for sections where samples are scarce. For this reason there has a been a shift in testate amoebae research from locally-specific transfer functions toward large spatial-scale collaborative datasets to encapsulate the longest possible gradients (e.g. Amesbury et al., 2016).

Following this, it is suggested that testate assemblages change on seasonal cycles, and therefore time of sampling is an important consideration which may hinder the development of suitable training sets intended to represent yearly-averaged environmental conditions. Rapid speciation in testate (Charman,

2001), and potential for optima-tolerances to shift through time, must also be considered when comparing living assemblages with those from fossil cores.

Ongoing debate questions how many individual testate amoebae should be counted in each sample. The optimum number balances the statistical validity and accuracy losses of counting too few, versus efficiency losses associated spending time counting too many. It is customary that testate amoebae based transfer function studies state clearly the number of tests counted in the methodology, and this highlights variability between authors. Payne and Mitchell (2009) study the balance between identification of main variability in diversity, and time efficiency, finding that 100 tests per samples is sufficient for redundancy analysis and transfer function development and therefore represents the most suitable compromise. If the aim of counting is to generate full taxon lists, or to identify minor but ecologically relevant community changes, it is recommended that counts are increased to 150 (Payne and Mitchell, 2009). However, conducting larger counts is often unnecessary if it reveals only a higher number of rare taxa unlikely to affect the environmental interpretation of the assemblage (Barnett et al., 2013) or if they will fall below the concentration limit set for subsequent analysis. It is also suggested that if overall taxonomic diversity is very low, counts of 50 tests (or even less) can provide robust test concentration and assemblage composition data (Royles et al., 2016). It can be summarised that the most suitable number varies depending on site specific characteristics, and overall aims of the study, although count totals of 100 should generally be targeted where possible (Ooms et al., 2011a).

In some archives test preservation (taphonomy) can hinder counting, with concentrations sometimes insufficient to permit analysis. Where a sufficient number of tests are available palaeoenvironmental interpretation may still be

limited by preservation differences between tests of different species (Swindles and Roe, 2007; Neville et al., 2010). For example, Roe et al. (2002) found poor preservation of delicate idiosomic tests (e.g. *Tracheleuglypha* spp.) in salt-marshes, and also found that sediment desiccation decreased preservation quality. Species of the genus *Euglypha* are frequently reported absent from sub-fossil peats even though they are found abundantly at the surface (Swindles and Roe, 2007). Ecosystem; acidity, moisture availability and nutrient status (Payne, 2009) have all been found to contribute to testate preservation variation. Swindles and Roe (2007) tested decay rates of tests in pH 0.3-0.5 solutions, and found a high degree of variation both between and within different genera. Although such pH values could not be found in peatlands the laboratory experiment essentially shows the long-term effect on tests deposited in acidic environments. A potential solution is offered by Booth and Jackson (2003) who excluded species not present below the acrotelm from the training set used to build transfer functions. Whilst testing for similarity between surface and fossil assemblages is beneficial, palaeoenvironmental data would be discarded needlessly if preferential preservation is not the true cause of species absence. This is particularly problematic in studies where diversity at the surface is already limited. The limits of preservation for the site investigated in this project are unknown.

It has been suggested that the extreme conditions on the sub-Antarctic islands may hinder test preservation, with freeze thaw conditions potentially destroying empty tests. However, testate amoebae have been used without the hindrance of poor preservation as palaeohydrological indicators from Northern permafrost peatlands which are frozen beneath a thin active layer (Swindles et al., 2015a, 2015b). The taphonomy of testate amoebae in sub-Antarctic peatlands is relatively poorly known, with no known studies specifically investigating this.

3.2.5. Testate amoebae in the Antarctic region

The following section synthesises existing records of testate amoebae specifically from the Antarctic region, updates previous reviews (e.g. Smith, 1996) with recently published data and covers the sub-Antarctic region for the first time. The aim is to summarise these diverse and sometimes ageing studies; to up-date maps of existing records, and include the sub-Antarctic region for the first time, to assess broad trends in testate amoebae communities and diversity, and identify opportunities for future research.

Testate amoebae communities are currently documented throughout the Antarctic region, and have been found in a range of environments including; peats, moss-banks, lakes, water-courses and soils (Fig. 4; Table. 3). However, the number of records from the Southern Hemisphere and particularly the Antarctic region remain less rigorously investigated compared to the Northern boreal and temperate regions (Reczuga et al., 2015). Spatially, the testate amoebae fauna of most of the larger sub-Antarctic islands have now been investigated. Recent work by Royles et al., (2013, 2016) on the Antarctic Peninsula has significantly improved our understanding of the species found there. Research led by Vincke (Vincke et al., 2004a, 2004b, 2006, 2007) has made a major contribution to records of fauna from Île de la Possession and also added to the known diversity of South Georgia.

A common feature across most existing studies is that they include only a small number of samples taken from a limited number of environments. It appears that sampling is often opportunistic due to the inherent difficulties of conducting fieldwork in the region, and is therefore often constrained to areas proximal to bases. This is problematic for diversity assessments, and hence it is expected

that the known diversity of the region will continue to rise as more in depth studies are undertaken. For this reason, repeat analysis is also required at most locations that have studied previously some of which have not been studied for over 100 years (e.g. Auckland, Macquarie and Heard Islands; Table. 3). Indeed, the inaccessibility of the only records from Heard Island (Richters, 1907) , prevented their inclusion in this review. In depth investigation (and re-investigation) of testate amoebae diversity from the sub-Antarctic is timely and necessary; it is expected that the current global extinction rates and losses of habitat will prevent discovery of new species (Pimm et al., 2014). This is of particular relevance to the sub-Antarctic peatlands, which are extreme, unique environments and therefore vulnerable to climate changes. The potential for sub-Antarctic peatlands to yield currently undiscovered testate amoebae species is substantial, owing to possible endemism (Kier et al., 2009) and the paucity of existing records.

Testate amoebae communities in the Antarctic region are generally characterised by a low diversity of cosmopolitan species, and overall concentrations are lower than at temperate latitudes. Euglyphid taxa (e.g. *Trinema lineare, Assulina muscorum* and *Corythion dubium*) are often dominant and ubiquitous; likely due to their wide ecological plasticity (Todorov and Golemansky, 1996) and suitability to maintain high reproduction-rates under harsh climatic conditions.

Recent studies reporting surface assemblages from previously investigated sites frequently find a higher diversity of species compared to earlier studies, generally attributed to limited sampling and potentially taxonomic uncertainty (e.g. Vincke et al., 2004a; Heger et al., 2009). The actual cause may be more complicated, in part, also related to rapid temperature rise forcing community restructuring and leading to increased diversity. The effect of temperature on testate amoebae on

the sub-Antarctic island remains unstudied, despite the possibility of obtaining unique palaeoclimate datasets. Research efforts in this area are required, it is suggested that such research could follow a similar method to Royles et al. (2013) using the concentration of testate amoebae to track microbial productivity thorough time, which can in turn be linked to temperature.

Microorganism cosmopolitanism is an area of current scientific debate with two main hypotheses (Heger et al., 2009). Testate amoebae are considered a model group of organisms in this field of study and the sub-Antarctic is a crucial region because oceanic separation between islands act as geographical barriers and facilitate endemism. Hypothesis 1 postulates that microorganisms are ubiquitous, and that their global distribution in suitable environments stems from their small size and abundance which allows continual dissemination on the wind, rafted on vegetation, and by adherence to birds (Smith and Wilkinson, 1986). Hypothesis 2 concerns biogeography and assumes that at least some microorganisms have a limited distribution due to their size (Smith and Wilkinson, 2007). Spatial analysis of testate amoebae supports both arguments, with the apparent existence of geographical barriers preventing the spread of larger (<100um (Smith et al., 2008)) and heavier species being postulated (Mieczan and Adamczuk, 2015). Evidence of potentially endemic species include; *Argynnia spicata* (Swindles et al., 2014) and *Apodera vas*, both found only in the Southern Hemisphere and in abundance on the sub-Antarctic islands (Smith et al., 2008). In contrast many species have an apparently cosmopolitan and ubiquitous distribution throughout the world. Suitable investigation of the biogeographic factors influencing testate amoebae diversity in the region is lacking, and a concerted and wide-scope strategy is necessary to fully address the debate.

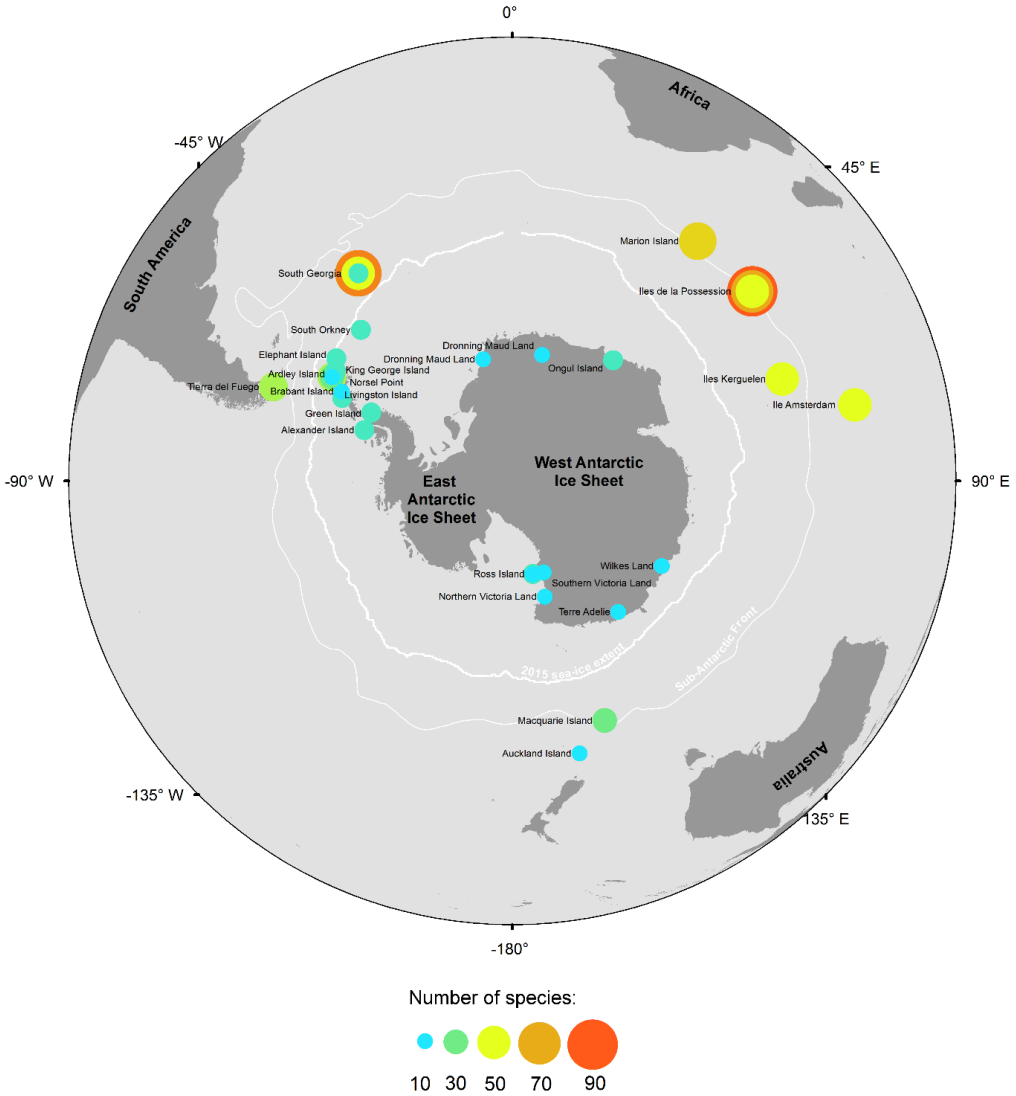

Figure 4 | Spatial distribution of testate amoebae studies in the Antarctic region. Information is plotted from Table 3. Note that in the interest of clarity the size of each marker and colour indicate the number of species reported in each study. Maximum sea-ice extent in 2015 (bold white line) and the sub-Antarctic front indicated by the thin white line.

The sub-Antarctic is an important region for this debate because; the strong westerly wind circulation, isolation of suitable habitats and latitudinal temperature gradient all contribute to increase the chance of endemism (ie species with a limited geographical distribution). Of specific interest to this study is *Argynnia* (Nebela) *antarctica*, currently found only on Marion Island (Grospeich, 1971). *A. antarctica* test morphology is similar to the cosmopolitan species *A. dentistoma* Penard (Heger et al., 2009) and without molecular data there is confusion whether it is a distinct taxon. Determining whether the species is indeed distinct and endemic is an important question because it could provide evidence supporting or contradicting the limited distribution of some testate amoebae.

Length and breadth ratio of tests analysed by Heger et al. (2009) are the only data currently available, and do not present any clustering of similarly sized individuals indicative of distinct species.

In addition to biogeographical controls, average yearly temperature (latitudinal position) has been theorised as a determinant of testate amoebae assemblages present in the Southern Ocean region. Recorded assemblages in colder (more poleward) areas are generally less diverse (Fig. 4). Smith (1982) showed that a diversity-latitude regression fitted to high Southern latitude diversity values predicted tropical diversity, which therefore suggested the presence of a latitudinal diversity gradient (Bobrov et al., 2015). However, more recent research (e.g. Vincke et al. 2006) in the sub-, continental-, and maritime-Antarctic has found a higher diversity than previously reported which potentially invalidates the theory. Indeed, it is likely that diversity variability across the Southern Ocean region is controlled by an array of different factors, including; biotic and abiotic population controls, dispersal rate, distribution mechanisms, and island size.

Insufficient sampling intensity due to inaccessibility of certain regions may artificially reduce the inferred diversity. Indirect effects of temperature also impact testate amoebae assemblages. For example, diversity of microclimates will vary with latitude, driven by factors such as; exposure, vegetation, soil type, available land area and topography (Vincke et al., 2006). Latitudinal position may also affect the precipitation levels which are variable across the region (Table. 1; Fig. 2). In close agreement with other regions, precipitation (moisture content) is also suggested as a significant control of species assemblages in the sub-Antarctic (Vincke et al., 2006) and is itself controlled by factors including peatland hydrology, temperature and precipitation type.

The climate of the sub-Antarctic islands is generally stable year round, due to oceanic proximity, but the average is cold. The low temperature has been suggested to produce a low dead-living test ratio, 2.2 (31%) on higher-latitude islands such as South Georgia compared to an average of about 10 in temperate regions (Vincke et al, 2006). It is suggested that empty tests are likely to be destroyed by water penetration and subsequent splitting by freeze-thaw cycles (Vincke et al., 2004a). This process could also lead to preferential preservation of certain species and therefore (1) complicate palaeoenvironmental interpretations or (2) reduce the amount of palaeoenvironmental information that it is possible to extract from testate amoebae analysis. Quantification of difference between living test diversity and fossil assemblages is therefore an essential factor of consideration before palaeoclimate application. The potential effects of preferential preservation of testate amoebae in cold climate environments, and more specifically the sub-Antarctic, is an area requiring further research. Currently the site investigated in this project does not experience permafrost conditions. Consideration must be given to the potential of such conditions dominating during colder climatic conditions in the past and temperature records for the Late Quaternary may need to be developed where they are not currently available, before palaeoenvironmental interpretations can be made. Ornithogenic soils (i.e. those influenced by proximity to bird colonies) are a feature of the islands in the Southern Ocean and authors report that testate amoebae do not occur in them; either living or fossil (e.g. Petz, 1997; Vincke et al., 2007). Fellfield soils, which have limited vegetation and occur in exposed areas, also have a generally low abundance and diversity of species compared to moss or freshwater habitats.

Table 3 | Selected records of testate amoebae diversity in the sub-, maritime- and continental- Antarctic regions ordered by increasing latitude southward. Number of species (Spp. No.) accounts for diversity identified in each study and not a compound diversity for each location. Preliminary accounts deemed inappropriate for assessing diversity are omitted for clarity, unless they are the only available record. Habitats sampled but devoid of testate amoebae species are marked with an asterisk. Column entitled 'palaeo- reconstruction' indicates whether the primary aim was to use testate amoebae assemblages to infer information about the climate or environment in the past.

Location	Latitude / Longitude	Habitats sampled	Spp. no.	Dominant testate amoebae species	Test Concentration (tests/dry g^{-1})	Palaeo-reconstruction?	Reference
Île Amsterdam	37°41'S, 77°31'E	Freshwater pools / rivers / moss	43	*Trinema lineare* *Assulina muscorum* *Corythion dubium*	-	No	Heger et al. (2009)
Île de la Possession	46°25'S, 51°59'E	Soil / ornithogenic soil*	65	*Trinema lineare* *Euglypha laevis*	-	No	Vincke et al. (2004a)
	46°24'S, 51°46'E	Pools / lakes / rivers	85	*Trinema lineare* *Trinema enchelys* *Difflugiella crenulata*	-	No	Vincke et al. (2004b)
	46°21'S, 51°42'E	Ornithogenic soil (Albatross colony)	44	*Trinema lineare* *Difflugiella oviformis* *Euglypha rotunda* *Microchlamys patella*	-	No	Vincke et al. (2007)
Marion Island	46°55'S, 37°45'E	Moss / vegetation	53	-	-	No	Grospietsch (1971)
Îles Kerguelen	49°24'S 69°10'E	Moss / vegetation	50	-	-	No	Bonnet (1981)
Auckland Island	50°41'S, 166°06'E	Moss	6	-	-	No	Penard (1911)
Heard Island	53°06'S, 73°31'E	Reference Unavailable					Richters (1907)
Tierra del Fuego	54°41'S, 68°29'E	Ombrotrophic bog	38	*Difflugia pulex* *Difflugia pristis*	-	Yes	van Bellen et al. (2015)
South Georgia	54°10'S, 36°40'W	Stream / lake / pool	46	-	-	No	Beyens et al. (1995)
	54°10'S, 36°42'W	Aquatic moss / terrestrial moss	71	*Trinema lineare* *Microchlamys patella* *Corythion dubium*		No	Vincke et al. (2006)

	54°20'S, 36°30'W	Soil / peat / moss / ornithogenic soil*	20	-	-	No	Smith (1982)
Macquarie Island	54°30'S, 158°57'E	Moss / Soil	23	-	-	No	Penard (1911)
Signy Island; South Orkney	60°43'S, 45°38W	Moss / soil / peat	18	*Corythion dubium*	-	No	Heal (1965)
South Shetland; Livingston Island	62°40'S. 60°30'W	Moss / soil / lake	24	*Trinema lineare* *Corythion dubium*	-	No No	Todorov and Golemansky (1996)
	62°39'S, 60°21'W	Moss / soil / aquatic	38	*Trinema lineare* *Euglypha rotunda* *Centropyxis aerophila* *Corythion dubium*	-	No	Todorov and Golemansky (1999)
	62.27°, 59.45°	Supralittoral marine sand	8	-	-	No	Golemansky and Todorov (1999)
South Shetland; Elephant Island	61°08'S, 54°42'W	Moss	9	*Corythion dubium* *Microcorycia radiate* *Assulina muscorum*	38,521 ± 50,436	Yes	Royles et al. (2016)
	61°12'S, 55°11'W	Moss / Mineral soil / moraine clay* / Ornithogenic soil*	15	*Pseudodifflugia gracilis* *Assulina muscorum* *Corythion dubium* *Phryganella acropodia*	-	No	Smith (1972)
South Shetland; King George Island	62°35'S, 45°30'W	Moss	15	*Trinema lineare* *Corythion dubium* *Centropyxis aerophila*	-	No	Mieczan and Adamczuk (2015)
South Shetland; Ardley Island	62°12'S, 58°56'W	Moss	6	*Corythion dubium* *Microcorycia radiate* *Assulina muscorum*	19,897 ± 18,119	Yes	Royles et al. (2016)
Antarctic Peninsula; Alexander Island	69°22'S, 71°50'W	Moss	12	*Corythion dubium*		Yes	Royles et al. (2013)
Antarctic Peninsula; Brabant Island	64°17'S, 62°20' W	Moss / vegetation	7	*Centropyxis aerophila* *Assulina muscorum*		No	Smith (1987)
Antarctic Peninsula; Norsel Point	64°45'S, 64°05'W	Moss	3	*Corythion dubium* *Microcorycia radiate* *Assulina muscorum*	21,001 ± 32, 368	Yes	Royles et al. (2016)
Antarctic Peninsula; Green Island	69°19'S, 64°09'W	Moss	7	*Corythion dubium* *Microcorycia radiate*	26, 253 ± 26,426	Yes	Royles et al. (2016)

				Assulina muscorum			
Ross Island, Antarctica	77°30'S, 168°00'E	Moss / Moss soil	3	*Diplochlamys timida* *Arcella arenaria* *Heleopera petricola*	-	No	Smith (1992)
	77°30'S, 166°00'E	Moss / lake	11		-	No	Penard (1911)
Antarctica; Wilkes Land	69°00'S, 120°00'E	Moss / Fellfield / Ornithogenic soil*	5	*Corythion dubium* *Assulina muscorum* *Schoenbornia viscicula (fellfield)*	14,386 ± 5694 (moss) 426 ± 129 (fellfield)	No	Petz (1997)
Antarctica; Ongul Island	67°30'S, 53°00'E	Moss	12		-	No	Smith (1992) *references within*
Antarctica; Dronning Maud Land	73°02'S, 13°24'W	Moss / lichen	2	*Corythion dubium* *Trgonopyxis arcula*	-	No	Smith (1992)
Antarctica; Dronning Maud Land	71°50'S, 04°00'E	Moss	2	*Centropyxis aerophila* *Corythion dubium*	-	No	Smith (1992)
Antarctica; Southern Victoria Land	78°02'S, 164°00'E	Moss	5	*Arcella arenaria* *Centropyxis aerophila* *Difflugia lucida*	-	No	Smith (1992) *references within*
Antarctica; Northern Victoria Land	71°15'S, 163°00'E	Fumarole heated ground	1	*Corythion dubium*	-	No	Smith (1992) *references within*
Antarctica; Terre Adelie	75°00'S, 139°00'E	Moss / pool	9		-	No	Decloitre (1960); Decloitre (1964)

0-20 21-40 41-60 61-80 81-100 species.

3.3. Reconstructing changes in Southern Hemisphere Westerly Wind behaviour

3.3.1. Introduction

Due to the importance of the SHWW-Southern Ocean-Atmospheric CO_2 coupled component of the earth system (Moreno et al., 2014), increasing scientific attention is paid to investigating how wind behaviour has varied since the Last Glacial Maximum (s*ee section 2.1*). The principle aim of these works is to generate a systematic understanding of wind variability, set within a precise chronological framework that is required for calibrating the models used to predict future changes (Varma et al., 2012) and to contextualise the short observational record with long-term natural variability. Exactly how the wind strength and geographical position has changed remains controversial and unstudied for large parts of the Southern Ocean region (Fig. 5). The paucity of reliable palaeodata is compounded by significant disagreement between existing records, and palaeodata-model inter-comparisons that frequently return contradictory results (Kohfeld et al. 2013). These uncertainties have been attributed to the limited number of palaeoclimate sites, the general clustering of sites toward the modern wind-belt periphery where changes are harder to detect, and the convoluted proxies used to develop reconstructions (Saunders et al., 2014). Combined, this fundamentally limits our ability to understand the mechanisms of past and future change in the climate-ocean-carbon dynamics that are driven by the winds (Turney et al., 2016b) (*see section 2.1)*.

Reliable palaeowind reconstructions in greater frequency, temporal and spatial resolution, are therefore required to advance our current understanding (Saunders et al., 2015). However, obtaining consistent, high-quality reconstructions is troublesome. The Southern Ocean region is characterised by a scarcity of land-areas, extreme isolation, and hostile climatic conditions. These

factors provide a challenging obstacle for research and assumingly explain the current scarcity of available palaeodata from the vast non-continental parts of the region.

Very few reliable palaeowind datasets currently originate from the sub-Antarctic islands, despite their location in the latitudes where present day westerlies peak in intensity (Fig. 5), however an expanding diversity of palaeoenvironmental techniques are now being developed and applied there. Owing to the rapidly changing field, existing reviews of palaeodata now require updating with recent advancements. Previous reviews have prioritised discussion of either specific time-periods or spatial regions, over the fascinating and diverse methods used to generate the reconstructions. Therefore, the purpose here is to evaluate the methods (i.e. locations, archives and proxies) that are used either within or close to the core wind-belt (~40-60°S). The review aims to: (1) summarise and explain the various proxy applications, interpretations, and ways different archives have been utilised, (2) discuss technique specific limitations, and (3) map the distribution of these existing palaeowind records from the region.

The following section is divided by discrete archive types, although it is acknowledged that many proxies are applicable to multiple archives. Selected examples of the applications of each method are described, and proxy interpretations for all of the studies included in this review are explained in Table. 4. In line with the rationale of this project, biological proxies (pollen, diatoms and sub-fossil wood) retrievable from peatland archives are granted increased attention. Because the scope of this project is of method development, analysis and discussion of reconstructed trends in wind behaviour are omitted here and instead the reader is directed to thorough reviews by: Kilian and Lamy (2012), Kohfeld et al. (2013) and Fletcher and Moreno et al. (2012).

Table 4 | Palaeoenvironmental techniques, archives and proxies used for reconstruction of Southern Hemisphere Westerly Winds over the Southern Ocean. Number refers to the site location shown in Fig. 5.

	Reference	Location	Longitude	Latitude	Archive	Proxy	Interpretation	Time Period (k yr B.P.)	Age-control (Number)
1	Turney et al., (2016b)	Campbell Island	169.080	-52.550	Sub-fossil trees	*Dracophyllum* tree-line altitude	Altitudinal limit (tree-line) of *Dracophyllum* is reconstructed and related to current extent on the island. Lower altitude is indicative of periods of greater exposure and lower temperature, which are linked to stronger westerly winds. Reconstruction based on fragments of wood eroding from peat sequences.	5.45 – 0	Radiocarbon (24)
2		Auckland Island	166.133	-50.772	Sub-fossil trees			5.79 – 0	Radiocarbon (8)
3	Musotto et al., (2016)	Terra Australis, Argentina	-67.764	-54.621	Peatland	Pollen and Fungi	Pollen and fungi microfossils are used to interpret changing ecological conditions linked to precipitation and therefore wind strength by present day correlation.	14.4 - 0	Radiocarbon (6) Tephra
4		La Correntina, Argentina	-66.983	-54.550	Peatland	Pollen and Fungi		15.2 – 0	Radiocarbon (7) Tephra
5	Turney et al., (2016a)	Falklands Islands	-57.785	-51.690	Peatland	Foreign pollen	Concentration of allochthonous pollen from South America increases with westerly wind strength and alignment.	2.6 – 0	Radiocarbon (9) Caesium-137
6			-57.785	-51.690	Peatland	Charcoal	Concentration of (foreign) microcharcoal increases due to enhanced burning (warmer Patagonian climate) and stronger westerly airflow. Charcoal count in size range <106 µm considered as wind transported (i.e. not produced locally).		
7	Vanneste et al. (2015)	Harberton Bog, Tierra del Fuego	-67.225	-54.876	Peatland	Atmospheric Dust Deposition	Quantification of atmospheric dust concentrations delivered to an ombrotrophic bog. Increased	16.2 – 0	Radiocarbon (10) Tephra -Hudson Volcano (1)

							concentrations indicate greater atmospheric dust transport and therefore stronger winds.		
8	Van der Putten et al., (2015)	Kerguelen Archipelago	69.000	-49.000	Peatland	Multiproxy	Climate based interpretation focusing on precipitation delivered by westerly winds and temperature.	15.8 – 10.9	Radiocarbon (20)
9	Saunders et al., (2015)	Campbell Island	169.134	-52.543	Lacustrine sediment	Diatoms	Salinity of waterbodies on the island is directly proportional to wind governed salt-spray inputs. Diatom assemblages will be used in subsequent research to reconstruct past salinity and therefore wind behaviour.	No reconstruction	Not dated
10	Saunders et al., (2009)	Macquarie Island	158.873	-54.601	Lacustrine sediment	Diatoms		No reconstruction	Not Dated
11	Saunders et al., (2014)	Macquarie Island	158.856	-54.648	Lacustrine sediment	Diatoms		Last Glacial Maximum to present.	-
12	Zimmermann et al., (2015)	Laguna Potrok Aike, Argentina	-70.378	-51.966	Lacustrine sediment	Diatoms	Diatom assemblages used to reconstruct lake level change, based on relative abundance of planklotic vs. benthic and epiphytic species. Higher lake level indicates more precipitation from easterly winds corresponding to periods of decreased SHWW strength.	15.6-10.51	Radiocarbon
13	Strother et al., (2014)	Annenkov Island	-37.062	-54.496	Lacustrine sediment	Non-native pollen deposition	Deposition of allochthonous pollen from South America increases with westerly wind strength.	7.0 – 0	Radiocarbon (32)
14	Herman & Brandon (2015)	Patagonian Andes	-73.500	-47.000	Glacial modelling	Glacial erosion	Modelled rates of glacial erosion that are proportional to temperature and precipitation over Patagonian Andes, and therefore indicate wind conditions in the past.	2-3 Ma – 0	
15	Van Daele et al., (2016)	Lago Castor, Patagonian Andes	-71.776	-45.592	Lacustrine sediment	Sediment geometry	Lacustrine seismic-reflection and sediment core studies used to infer changes in wind strength. Westerly	41.7 – 0	Radiocarbon (13)

						airflow sets-up currents within the lake and therefore sediment patterns reflect westerly wind variability through time.		
16	Lisé-Pronovost et al., (2015)	Laguna Potrok Aike, Argentina	-70.390	-51.960	Lacustrine sediment	Rock magnetism	Magnetism measurements used to infer dust deposition rates, where increased dust concentrations indicate stronger winds.	51.2 – 0	Radiocarbon (58)
17	Schimpf et al., (2011)	Bahia Arevalo, Chile	-72.711	-52.404	Speleothem	Growth rate and stable isotopic ratios	Speleothems used to reconstruct temperature and precipitation, and then related (assuming present day correlation) to wind velocities.	4.5 – 0	$^{230}Th/^{234}U$
18	Tonello et al., (2009)	Cerro Frias, Argentina	-72.696	-50.403	Peatland	Pollen	Quantitative reconstruction of southern South American annual precipitation and by association changes in westerly wind.	12.4 – 0	Radiocarbon (5)
19	Mayr et al., (2007)	Patagonia, Argentina	-70.390	-51.960	Lacustrine Sediment	Allochthonous Pollen flux	Long distance transport of pollen from forests in the Andes deposited in the Patagonian-steppe region, where increased forest pollen flux indicates periods of stronger wind.	11.6 – 0	Radiocarbon (13) Tephra (2)
20	Björck et al., (2012)	Isla de los Estados, Tierra del Fuego	-64.617	-54.818	Peatland	Aeolian sand influx	Concentration of wind-transported sand grains within the record used to infer palaeowind intensity.	14 – 0	Radiocarbon (19)
21	Anderson et al. (2009)	Southern Ocean (Atlantic;TN057-13-14)	05.100	-53.200	Ocean Sediment	Biogenic Silica	Quantity of biogenic silica production used to indicate the wind-driven upwelling of nutrient rich water in the southern ocean. Increased biogenic silica within the records indicate periods of greater primary production at the ocean surface when more intense westerlies drove stronger upwelling.	~80 – 0	-
22		Southern Ocean (Pacific;E27-23)	155.240	-59.620					
23		Southern Ocean	-170.000	-61.900					

		(Pacific;NBP9 802-6PC)							
24	Moy et al., (2008)	Lago Gaunaco	-72.803	-51.871	Lacustrine sediment	Stable isotopes	Reconstruction of lake level changes using stable carbon and oxygen isotope ratios, and relating this hydrologic regime to wind behaviour.	1.8 – 0	Radiocarbon (6)
25	Mayr et al., (2013)	Patagonia, Argentina	-70.390	-51.960	Lacustrine Sediment	Oxygen isotope ratios of aquatic cellulose ($\delta^{18}O_{cell}$)	$\delta^{18}O$ values taken from aquatic moss cellulose (plant debris) is used to indicate past lake-water isotopic values ($\delta^{18}O_{lake}$). Evaporation and inflow rates control $\delta^{18}O_{lake}$ and are linked with lake level change. Strength of the westerly winds is proportional to precipitation at the location, and therefore governs lake water-balance.	16.7 – 10.7	Radiocarbon
26	Saunders et al., (2012)	Rebecca Lagoon, Tasmania	144.695	-41.192	Lacustrine Sediment	Scanning Reflectance Spectroscopy	Reconstruction of precipitation based on scanning reflectance spectroscopy outputs and by association westerly wind strength and latitudinal position	3 – 0	Radiocarbon (8) Lead-210
27	Echeverria et al., (2014)	Avellaneda Peninsula	-72.864	-50.275	Peatland	Pollen Charcoal Sedimentation	Multi-proxy analysis of precipitation changes and then reconstruction of westerly wind conditions by association with modern day correlation.	5.15 – 0	Radiocarbon (4)
28	Moreno et al., (2010)	Lago Guanaco	-72.802	-51.871	Lacustrine Sediment	Pollen Charcoal	Changing composition and position of an ecotone is related to precipitation conditions. Precipitation strongly correlated with westerly wind strength in the region, allowing reconstruction of wind behaviour.	14 – 0	Radiocarbon (17) (High resolution; 50 yr)
29		Lago Condorito	-73.158	-41.855	Lacustrine Sediment	Pollen Charcoal		14 – 0	Radiocarbon (18)
30	Villa-Martínez & Moreno (2007)	Vega Nandu, Chile	-72.776	-51.006	Peatland	Pollen and Charcoal	Pollen and charcoal records linked to variations in precipitation of westerly origin and therefore linked to wind conditions.	12.6 – 0	Radiocarbon (12)

31	Moreno et al., (2009)	Lago Guanaco	-72.802	-51.871	Lacustrine Sediment	Pollen and Charcoal	Changes in vegetation and fire frequency at the Patagonian forest-steppe ecotone, linked to precipitation and westerly airflow by present day correlation.	3.8 – 0	Radiocarbon (10)
32		Pantano Margarita	-73.113	-51.127	Peatland	Pollen and Charcoal			Radiocarbon (3)
33	Whittaker et al. (2011)	South Island, New Zealand	171.459	-41.950	Speleothem	Growth rate and stable isotopic ratios	Speleothem growth is proportional to precipitation changes which are linked to westerly wind behaviour. Slow growth rate and enriched isotope ratios indicate periods of weaker westerly airflow.	73 – 11	Uranium series (18)
34	Putnam et al., (2010)	South Island, New Zealand	170.031	-44.017	Glacial landscape	Ice extent	Exposure dates of moraine features to measure the timing of glacier expansion on both sides of the Southern Alps. Climate deterioration causing glacier advance is related to Northward movement of the sub-tropical front (and westerly wind belt displacement).	14 – 12	^{10}Be exposure dates
35	McGlone et al. (2010)	Campbell Island	169.080	-52.550	Peatland	Pollen	Divergence between land surface temperature and sea surface temperature is used to infer changes in winds. Land warming relative to sea surface cooling is related to southward wind-belt migration and intensification, bringing more atmospheric heat southwards and driving increased upwelling of cold deep-water.	17.5 – 0	Radiocarbon
36	Lamy et al., (2010)	Seno Skyring, Patagonia	-71.772	-52.637	Ocean sediment	Clay/Silt Ratio	Clay/silt ratios of sediment act as a proxy for wind-induced long-distance clay transport	13 – 0	Tephra (4)
37		Bahia Beaufort, Patagonia	-73.648	-52.789	Ocean sediment	$CaCO_3$	$CaCO_3$ accumulation rates used as a proxy for palaeosalinity. $CaCO_3$ is produced by benthic and planktonic foraminifera that reflects relative influence of Pacific water at the core site. Increased westerlies bring more precipitation (lower salinities	13 – 0	Radiocarbon (2) Tephra (4)

						at the core site). Periods of low wind weaken the low-salinity surface layer.		
38		Magellan Strait, Patagonia	-73.781	-52.892	Ocean sediment	Siliciclastic material Terrestrial OC	Accumulation rate of terrigenous siliciclastic material and terrestrial organic carbon as a proxy for precipitation in the past.	13 – 0	Radiocarbon (4) Tephra (2)
39		Tamar Lake, Patagonia	-73.803	-52.905	Lacustrine sediment	Terrestrial OC	Accumulation rate of terrestrial organic carbon as a palaeoprecipitation proxy.	13 – 0	Radiocarbon (14) Tephra (2)
40		Munoz Gamero Peninsula, Patagonia	-72.923	-52.816	Peatland	Pollen	Pollen assemblage change through time as a precipitation proxy. Quantification of hydrophilic plant species as an indicator of stagnant water (small ephemeral lakes) during prolonged wetter periods.	13 – 0	Radiocarbon (9) Tephra (2)
41	Lamy et al., (2001)	Chilean Continental slope	-74.414	-41.052	Ocean Sediment	Sediment geochemistry	Interpretation predominantly based on iron content which reflects changes in weathering associated with rainfall variability.	7.7 – 0	Radiocarbon (7)
43	Knudson et al., (2011)	Milford Sound, New Zealand	167.92	-44.672	Fjord sediment	Sediment C:N ratio and $\delta^{13}C$	Ratio between terrestrial and marine organic carbon matter. Greater	1.34 - 0	Radiocarbon (3)
44		George Sound, NZ	167.376	-44.942	Fjord sediment		content of terrestrial than marine organic matter in cores indicates	1.88 - 0 / 1.74 - 0	Radiocarbon (6) / (3)
45		Thompson Sound, NZ	166.981	-45.239	Fjord sediment		periods of increased precipitation. Precipitation is linked to SHWW conditions in western New Zealand due to its high axial range.	3.71 – 0	Radiocarbon (3)

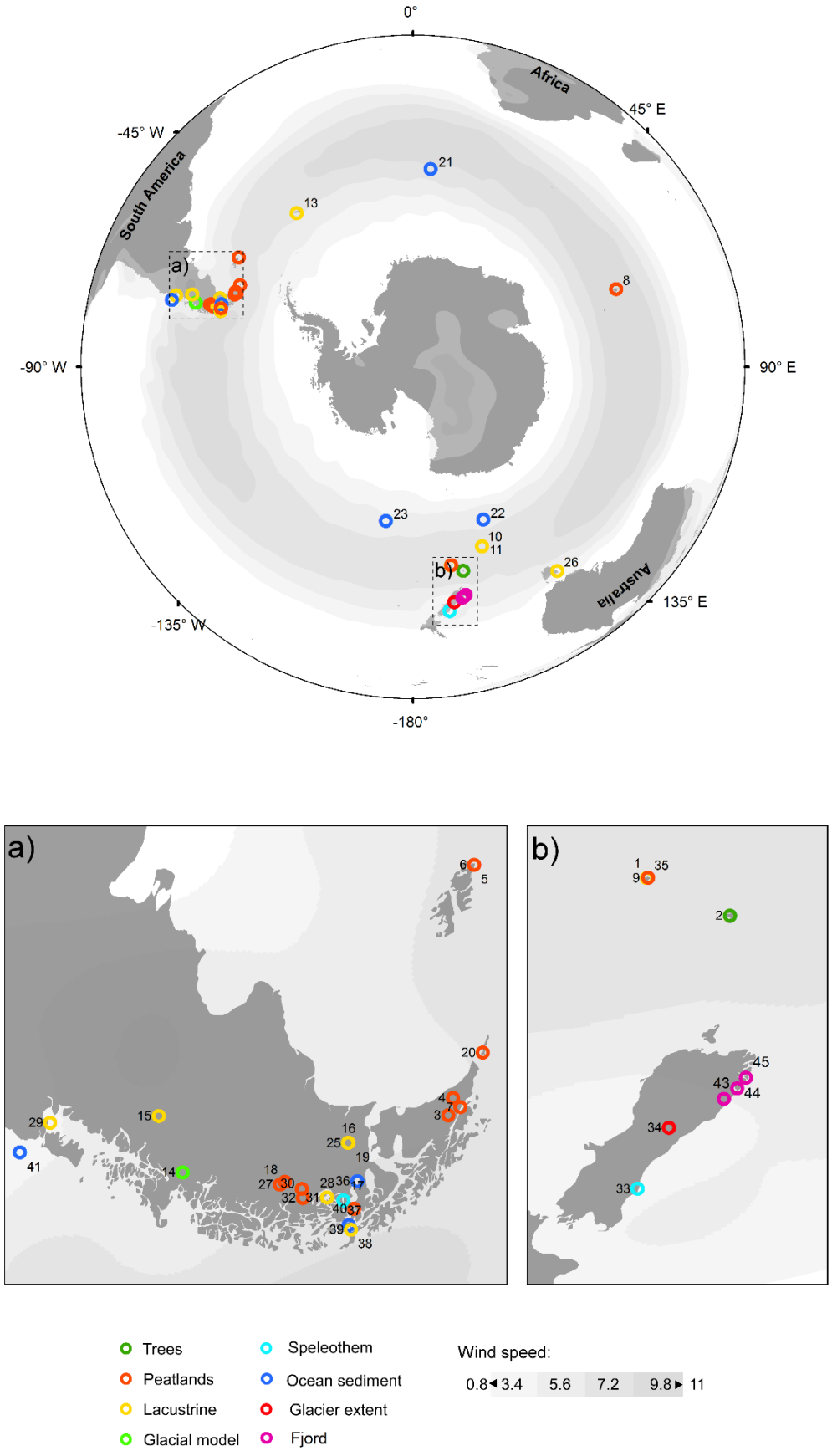

Figure 5 | Archives used for SHWW reconstruction from within or close to core belt (40-60°S). Inserts showing Patagonia and the Falkland Islands (a) and the south island of New Zealand (b). Note that where reconstructions using multiple cores are included, a separate point is shown for each location. Numbers refer to records presented in Table 4. Westerly wind belt shown using 20 Century NCEP re-analysis data of 1000 mb zonal wind (composite mean) from 1982-2012 (Kalnay et al., 1996; NOAA, 2016). Only the main wind field is depicted.

3.3.2. Peatlands and Lacustrine Archives

3.3.2.1 Pollen and charcoal

Pollen grains produced by phanerogam plant species disperse widely, and preserve well within sedimentary archives including lakes and peatlands throughout the southern high-latitudes. Identification of fossil pollen (palynology) from these sequences can be used to reconstruct vegetation patterns through time, and past environmental conditions can be inferred by relating them to modern analogues. Globally, palynology is one of the most useful methods in palaeoclimate research (Van der Putten et al., 2012b), and hence the potential applications to reconstruct the SHWW have attracted substantial interest. Use of pollen for wind-behaviour reconstruction can be divided into two key approaches; (1) reconstruction of climatic conditions linked to wind behaviour, and (2) quantification of long-distance aeolian-transported pollen deposition rates.

Reconstruction of climate

Southern South America is viewed by many workers as an ideal region to examine westerly wind variability throughout the SH mid-latitudes. Primarily this is because precipitation and climate are tightly coupled to wind behaviour (e.g. Garreaud, 2007; Harberzettl et al., 2007; Mayr et al., 2007; Moy et al., 2008; Lamy et al., 2010; Kilian and Lamy, 2012; Moreno et al., 2014). It is also the only continuous continental landmass inside the core wind-belt, and spans sub-tropical to sub-Antarctic environments (*see* Fig. 1; Moreno et al., 2010). This allows access to the abundance of suitable archives, and also permits investigation of latitudinal migrations of the wind-belt over time (Moy et al., 2008; Lamy et al., 2010).

Tight coupling between wind and precipitation occurs because the Andes Cordillera barriers westerly airflow, which drives intense orographic precipitation that is principally governed by low-level zonal wind-speed (Fig. 6). Correlation is strongly positive on the hyper-humid Pacific coast; the Andes and western fjords experience >4000 mm yr^{-1} precipitation and comparatively low rates of evaporation (Kilian and Lamy, 2012). Weather station data indicate a strong,

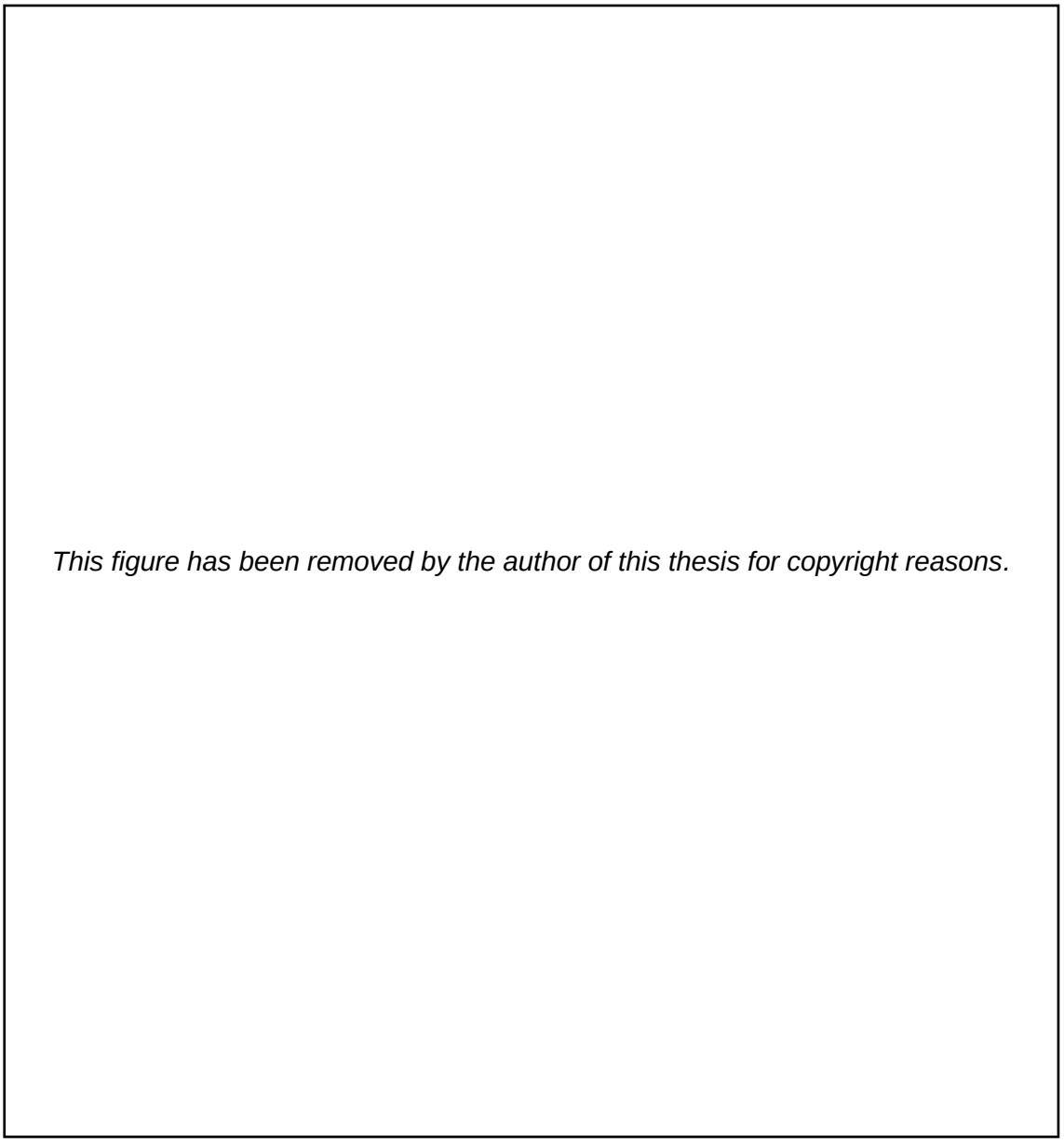

Figure 6 | Present day correlation between monthly precipitation anomalies and zonal wind strength at 850 hPa (a) **and vegetation in southern South America**. Demonstrates the strong positive correlation in western Patagonia, that is frequently exploited in wind-behaviour reconstructions. Dotted lines indicate regions where annual precipitation is in excess of 1,000 mm (Source: Garreaud, 2007). b) west-east transect through southern south America showing correlation between SHWW and precipitation along with other climatic variables that impact the regions vegetation (Source: Kilian and Lamy, 2012). c) generalised vegetation map showing the distribution of plant communities and precipitation gradients in southern south America (Source: Moreno et al., 2009 *modified from* Villa-Martínez and Moreno, 2007).

positive wind-precipitation correlation at the continental margin and within the Andes (R= 0.4-0.8; Kilian and Lamy, 2012).

Further East the climate becomes arid and correlation weakens (Fig. 6b; R= 0.4-0.2; Kilian and Lamy, 2012). Downwind of the Andes, within the Patagonian steppe, moisture depleted winds drive evaporation and produce a negative water balance (Garreaud, 2007; Moy et al., 2008; Moreno et al., 2010; Echeverria et al., 2014; Hulton et al., 2002). Precipitation in these regions falls to <500 mm yr^{-1} (Fig. 6c).

The strong precipitation, altitudinal and latitudinal gradients provide a distinctive and highly sensitive biological system for detecting hydrologic changes from pollen assemblages (Moreno et al., 2010). Broadly, vegetation ranges from magellanic moorland in the west to semi-desert Patagonian steppe in the east (Fig.6c; Kilian and Lamy, 2012). As well as the vegetation patterns, regional precipitation changes are also recorded in an array of records including palaeofire and lake level fluctuations (Moreno et al., 2014).

For palaeoclimatology, the observed correlations allow wind changes to be inferred (albeit indirectly) from precipitation reconstructions. Moreno et al. (2010) use pollen counts to document shifts in the composition and geographic position of the Magellanic deciduous forest-Patagonian steppe, and North Patagonian rainforest-Valdivian rainforest ecotones, interpreting shifts toward Patagonian steppe or Valdivian rainforest taxa as an indicator of drier conditions and therefore weaker SHWW. Previously, Moreno et al. (2009) used the same technique, by implicating the ratio of *Nothofagus*-Poaceae pollen to investigate precipitation-induced changes in forest-steppe vegetation respectively. Hydrological reconstructions from palynology in southern South America are

frequently reported (Table. 4), and are among the most important sources of palaeowind data currently available.

However, due to the complex pattern of correlation and landscape heterogeneity, consideration of site selection is crucial in obtaining suitable records and interpreting them correctly. Assumptions that vegetation assemblages provide a direct link to precipitation are also problematic, and that this relationship is constant through time, is debatable. Plants record a bioclimatic precipitation signal (i.e. growing degree-days, temperature and seasonal available moisture), and therefore it is uncertain whether reconstructed changes reflect precipitation and/or temperature conditions more strongly (Saunders et al., 2015). Temperature, does not display the same strong correlation with SHWW conditions and therefore it can hinder interpretations. It also makes the results difficult to interpret alongside other terrestrial moisture proxies, which record slightly different signals not always directly reflective of precipitation (Kohfeld et al., 2013).

It is also noteworthy that modern wind-precipitation correlations are assumed to be stable through time, and that the same trend also holds for regions currently outside the main wind-belt. An example of how the wind-precipitation correlation could have changed was recently highlighted by Van Daele et al. (2016). They explain that the enhanced orographic effect of the Patagonian Ice-sheet, which covered the crest of the Andes (Hulton et al., 2002) until ~20 kyr. B.P. caused an enhanced precipitation deficit and consequently more extreme arid conditions in the lee of the Andes, strengthening the precipitation gradient across the continent relative to the present day. More broadly, it must be hypothesised that changes in the global hydrological cycle during glacial to interglacial periods had a

substantial impact on plant available moisture patterns in the region, irrespective of wind conditions.

It may therefore be summarised that although pollen based precipitation reconstructions of the SHWW derived from South America have aided initial exploration of the subject, their wider application is potentially limited. The proxy is convoluted so potential error is introduced with each logic step that separates wind from the reconstructed variable. Palaeoclimate sites located along the precipitation gradient differ so considerably that they may require different proxies, and therefore interpretations may not be directly comparable.

Traditional palynology for wind reconstruction has also been attempted on sub-Antarctic South Georgia and Île de la Possession, however success is limited because pollen production is limited by the low diversity of vascular plant species that are present (25 spp., Van der Putten et al., 2004). A greater diversity of bryophyte species are present, and although they respond sensitively to environmental change (Van der Putten et al., 2008), they do not produce pollen and are therefore omitted from the record. Additionally, pollen of the common vascular species *Juneaceae* does not preserve well, further reducing species present in the long-term pollen record. In combination these factors mean that pollen based studies yield limited palaeoclimatological data (Van der Putten et al., 2004), and are therefore unable to detect low-magnitude climate events (Van der Putten et al., 2012b). Consequently, application of the technique for reconstructing westerly wind conditions is unlikely to yield reliable reconstructions covering the Late Quaternary period. Instead, macrofossil analysis, which records changes in both bryophyte and phanerogam vegetation, are frequently used in preference (Van der Putten et al., 2004, 2008, 2009).

Pollen based palaeohyrological studies are often supported with charcoal records (e.g. Echeverria et al., 2014; Villa-Martínez and Moreno, 2007), which can be used to reconstruct changes in fire regimes driven by changes in moisture availability over multi-millennial time-scales (Fletcher and Moreno, 2012).

Deposition of long-distance transported pollen grains

Quantifying deposition of non-native aerially transported pollen grains is a technique with potential to provide more reliable palaeo-wind reconstructions (Saunders et al., 2015: Strother et al., 2014). It provides a straightforward link with wind-behaviour and could therefore be considered a pure wind proxy. The method is advantageous because it is more independent from the temperature and precipitation conditions that hinder pollen based hydrologic reconstructions (Saunders et al., 2015), and can also overstep the limitations associated with traditional palynology on the sub-Antarctic islands.

Proxy interpretation follows that SHWW strength and latitudinal position principally control the quantity of pollen grains transported from an (up-wind) source region to a remote archive that is aligned to the prevailing wind. Long-distance transported grains are easily identifiable if they are non-native to the archive in which they are deposited. For example, *Nothofagus* pollen found in the Falkland islands records must originate from elsewhere (temperate South America), since the species has not grown on the islands throughout the time-period that the records span (Turney et al. 2016a). Quantification of shifts in grain deposition thereby provides a palaeowind proxy, where periods of low deposition are indicative of weaker and/or more poorly aligned airflow and vice-versa. The technique is also used in southern South America (e.g. Markgraf et al., 2003;

Moreno et al., 2009) and is one of the few palaeowind proxies currently applied on the sub-Antarctic islands (Table. 4).

Another example from southern south America is Mayr et al. (2007) who record non-native pollen in the sediment of Laguna Potrok Aike situated within the Patagonian-steppe in the lee of Andes. Non-native pollen from Andean forest taxa (AFT) can be separated from the local Patagonian steppe taxa and as a result increases in AFT provide an indication of stronger westerly airflow. However, the applicability of the technique in this region is limited temporally to the Holocene, because vegetation (pollen) assemblages were influenced more strongly by extreme temperature variability (McCulloch et al., 2000) and successional processes during deglaciation of the Patagonian ice-sheet (*as mentioned above*) than by wind regime thus complicating the record (Mayr et al., 2007). Turney et al. (2016a) identified influxes of South American pollen taxa to infer periods of strengthened westerly winds over the Falkland islands and the same technique was also investigated by Strother et al. (2014) on Annenkov Island, South Georgia. The results of foreign pollen counts presented by Turney et al. (2016a) must however be interpreted with caution. This is because the peatlands on the Falkland islands are prone to intense erosion by the westerlies, and consequently old pollen from eroded peat sequences is being remobilised and redeposited on the surface (Hodgson, pers. comms.). Turney et al. (2016a) use charcoal counts to support their interpretation, and find that peaks in foreign pollen coincide with peaks in charcoal. These charcoal particles are mainly of the size fraction <50 µm, equivalent in size to the pollen grains, and therefore suggest that they too have been transported by the wind from Patagonia.

Site selection is a major limitation of the technique in the sub-Antarctic; the continental pollen (or charcoal) source should be located proximal to the archive so that a high concentration is received but sufficiently isolated so that inputs are not constant through time (Turney et al., 2016a). This means that there are relatively few suitable regions and therefore the technique has potentially restricted applicability in the sub-Antarctic. Preliminary counts from Annenkov Island, South Georgia (Strother et al. 2014) and Île de la Possession (Van der Putten et al,. 2012a), both reveal very low concentrations of non-native pollen. Latitudinal position of the source relative to the archive is also an important concern. For example, on Campbell and Auckland islands non-native pollen influx would record meridional (North-South) and not zonal (West-East) changes in the wind field.

The technique is further limited by the assumption of constant atmospheric circulation patterns through time. Uncertainty can arise when using isolated island 'point-sites', where a hypothetical decrease in foreign pollen could indicate either less intense winds or a latitudinal shift of the core belt. In such cases records from other latitudes, which may not be complimentary, are needed to isolate the signals and this can complicate the interpretation. Finally, the source of foreign pollen is assumed constant through time and therefore can only be applied for species such as *Nothofagus* which have a well-known history of stable high abundance (Turney et al., 2016a).

3.3.2.2. Dust

Deposition of aeolian transported particles (e.g. dust and tephra) into a remote down-wind archive from a known source region, is another relevant proxy for SHWW reconstruction. Analysis of particle size can provide further information,

with the assumption that larger particles require stronger winds to transport them. Peatlands, especially raised ombrotrophic bogs, provide ideal archives for this purpose because they receive only atmospheric inputs of particles. Björck et al. (2012) incorporate a measure of Aeolian sand influx as a SHWW proxy into a multi-proxy analysis of a peat bog on Tierra del Fuego. They count particles in the size fractions 125-350 µm, 350-700 µm and >700 µm using a microscope, which provides a measure for the extreme wind conditions that are needed to aerially transport these large particles. The source of particles for this study are beaches, Quaternary age tills and exposed bedrock, which were located around the study site. Separation of the particles into different mineral types, allowed the individual sources to be identified, and can be used as a crude measure of relative wind direction in the past.

Also working from an ombrotrophic peatland archive in Tierra del Fuego Vanneste et al. (2015), measure accumulation rate of mineral dust from concentrations of rare earth elements within bulk peat samples. They also measure neodymium isotopic composition to trace the origin of the atmospheric dust. The authors suggest that the region surrounding the study site had available dust (fine glaciogenic sediments) and suitable conditions for atmospheric transport, throughout the time-period covered by the peat record. They find that dust deposition increased during the Antarctic Cold Reversal and late Younger Dryas, and attribute this to strengthened westerlies. Credibility of the record is leant by the same conclusions being drawn be other studies using different proxies in different areas (e.g. Mayr et al., 2013).

As mentioned above, peatlands of the sub-Antarctic are not ombrotrophic, and therefore this may limit the potential future application of this technique in the

region. Further, the need to identify the source of particles makes this analysis relatively time consuming. Kohfeld et al. (2013) discuss more general limitations, they suggest that dust deposition actually reflects a suite of factors in addition to transport by the wind. These include; the atmospheric residence time of particles, aridity and areal extent of the source region, trajectory of the winds and temporal changes in the availability of glaciogenic and glacial outwash sediments. Similarly, the particle size distribution also reflect a variety of factors, not just wind strength. These factors include; distance from source area, distribution of particle size at source, and proportion of dry to wet deposition of dust (Kohfeld et al., 2013). Therefore it can summarised that although dust based techniques provide a direct link with past wind conditions interpretations of the records must be treated with caution, and should be considered as part of the wider (regional) palaeoclimate picture (Kohfeld et al., 2013).

3.3.2.3. Diatoms

Diatoms are unicellular photosynthetic algae with a siliceous cell wall that often preserves well in lacustrine sediments. They have a high sensitivity to a range of environmental conditions (Fernandez et al., 2013; Soininen, 2016), due to their narrow ecological optima-tolerances, which make them useful for reconstructing environmental changes (Zimmermann et al., 2015). They occur in both salt and freshwater environments and have been used to reconstruct conditions including: nutrients, pH, temperature, water depth and additionally they also respond to changes in conductivity.

Zimmermann et al. (2015) used diatoms to infer hydrologic changes from lake level fluctuations at Laguna Potrok Aike. Unlike the precipitation delivered by westerly winds to the Pacific coast the hydrology of this lake is related to

precipitation brought by easterly airflow over the region during periods of weaker SHWW, and therefore the proxy works via anti-correlation between the westerlies and precipitation (Lamy et al., 2010). The proxy interpretation is based on peaks in planktonic diatom populations that coincide with increased lake levels (and weaker westerly winds). Fernandez et al. (2013) have also used diatom taxa indicative of lake level, ice-cover and turbidity to reconstruct precipitation and airflow over Isla de los Estados, an island in southern Tierra del Fuego.

Similarly to the use of pollen for palaeohydrological reconstruction, diatom proxies are limited by the convoluted linkage between lake level and wind behaviour. Indeed, the quality of the resultant reconstruction obtained through application of this methodology is limited by the uncertainty in this correlation. Therefore, an alternative use of diatoms has been proposed as a method to generate reconstructions of greater reliability. Saunders et al. (2009, 2015) have related specific environmental variables to diatom assemblages in lakes on sub-Antarctic Macquarie and Campbell Islands. They produce transfer functions that can be used to reconstruct past conditions linked to wind behaviour when applied to a sediment sequence.

Strong gradients of salinity in freshwater lakes and ponds (west-east) across sub-Antarctic islands have been recorded by numerous studies (Saunders et al., 2015). The principal control of salinity on the island water-bodies is salt-spray delivered from the ocean, corroborated by oceanic equivalent ratios of ions within them (i.e. $Cl>SO_4>Mg>Ca>K$) (Saunders et al., 2015). Salt-spray inputs are regulated by the strength of the westerly winds and consequently conductivity declines exponentially with decreasing exposure (i.e. distance to the west coast and altitude). These modern-day spatial changes therefore equate to wind-

strength regulated temporal variation in west-coast lake conductivity. Identification of diatoms preserved in lake cores from the west-coasts therefore provides a proxy for past westerly wind intensity (Saunders et al., 2009, 2015).

Saunders et al. (2014) used a diatom-conductivity transfer function developed for Macquarie Island to provide the first reconstruction of SHWW strength within the core-belt, from a record reaching back to the Last Glacial Maximum. Whilst the technique is still in its early stages, the results indicate stronger westerlies during the glacial period, followed by an abrupt decrease, and generally weaker winds through the Holocene. The results mirror dust flux and CO_2 concentration records from Antarctic EPICA Dome C, lending credibility to the diatom based reconstruction and highlighting the substantial potential of this technique.

Work is ongoing to apply the diatom-conductivity transfer function to lake sediment cores from the western coast of Campbell island, which is likely to yield a further direct reconstruction of SHWW strength in the near future. A diatom-based proxy of this type has significant potential to increase understanding of changing westerly wind behaviour. Utilisation of the lacustrine sedimentary archives provides opportunity for high accuracy dating and represents a high-resolution archive. In addition, lakes are accessible archives in the context of the sub-Antarctic, and are abundant across the main sectors. Diatom sampling from lakes is also time, labour and equipment efficient.

A potential issue in the application of this methodology is that lake volumes vary. Therefore, assuming uniform salt-spray deposition the salt signal in larger lakes will be less than smaller ones due to greater dilution. Whilst this is not a problem for the development of a transfer function, it may prove difficult when developing a reconstruction, especially if cores from multiple (different sized) lakes are used.

For example, given uniform salt inputs, the conductivity of a small volume lake will increase more rapidly than a lake with larger volume, altering the diatom community response to the environmental change.

Current debate surrounds the cosmopolitanism of microorganisms in the sub-Antarctic region. Although differences in diatom assemblages were reported between Campbell and Macquarie islands (Saunders et al., 2015) this is unlikely to represent a limitation of the technique but highlights the need for careful development and application of site specific transfer functions. Diatoms have been recorded widely across the Southern Ocean region, and therefore the technique has potential applicability over geographical areas including the core wind-belt. It is therefore suggested that this technique holds significant potential to address the question of symmetry in wind changes.

There are however, limitations associated with transfer function performance. A trend in the residuals of the model developed on Campbell Island means that it may over-estimate low-end conductivity and under-estimate high-end values, limiting the accuracy of output reconstructions and the sensitivity of the method. Poor diatom preservation is a further limitation, both in the assembly of transfer-functions and in the study of sub-fossil assemblages. Saunders et al. (2015) report that diatoms were absent in twelve of the original fifty-four samples and diatoms were poorly preserved in a further ten. It is currently unknown whether such issues will also hinder down-core reconstructions, and accurate quantification is necessary. This will be further complicated by the potential for preferential preservation where certain species may be omitted from the record leading to potentially erroneous interpretations.

3.3.2.4. Trees

The biological archive provided by tree growth is a newly applied method reliant on the sensitivity of ligneous plants to temperature and exposure induced by over-land westerly airflow. Specific ecological sensitivity results in a certain spatial colonisation pattern that is directly related to westerly wind conditions.

Tree-line reconstruction as a palaeowind proxy has been developed and applied exclusively for the evergreen angiosperm species *Dracophyllum* on Auckland and Campbell islands (Fig. 5) in the south-west Pacific (Turney et al., 2016b). Growth of *Dracophyllum* is highly sensitive to perturbations of soil drainage and exposure to the westerly winds. Sheltered areas (east facing hillsides, low altitude areas and gullies) on both islands support an abundance of *Dracophyllum*, which reach a maximum of 5 m in height. Conversely exposure (high wind speeds) and corresponding low temperatures on west facing slopes exert a deleterious control on colonisation. Dated fragments of subfossil *Dracophyllum* recovered from eroding peat deposits in more exposed (higher elevation) positions above the current tree-line are indicative of periods when wind influence decreased (Turney et al., 2016b). Use of the *Dracophyllum* extent for palaeowind reconstruction is also advantageous as a direct proxy with a single link between exposure and growth. Reconstructions using this method are a beneficial addition to existing records, and particularly suited to these islands where other direct proxies (e.g. deposition of non-native pollen) are not applicable.

One crucial limitation of the method is the chronological framework which means that short (e.g. decadal) timescale changes cannot be isolated from longer-term signals. Rather than applying a dated stratigraphic sequence to contextualise measured environmental changes, this approach uses dated fragments, exposed

by erosion, and collected at the surface from different locations above the current tree line. The assumption of older material with depth used in studies of vertical cores is inapplicable, and therefore hiatuses caused by repeated up and downslope migrations of *Dracophyllum* complicate the chronology of the record. Such overprinting coupled with the sampling strategy leaves only major trends at the extremes of environmental variability distinguishable and prevents estimations of the timing of specific lower-magnitude fluctuations.

Unlike stratigraphic studies the sample collection and data processing used by Turney et al (2016b) results in a discontinuous record of tree line with data-gaps where no individuals are sampled within specific age bands. The interpretation of such gaps is difficult and may indicate either error in the sampling strategy (i.e. an insufficient number of samples) or the deleterious effect of intense winds on *Dracophyllum* colonisation during the time-period. Traditional pollen records derived from the peatland archive are needed to indicate relative *Dracophyllum* cover during such periods to corroborate interpretation of the data (McGlone et al., 2010).

Tree line reconstruction is also limited to a narrow latitudinal band of applicability in the sub-Antarctic region. Campbell island at 52°S has the most southerly *Dracophyllum* colonisation, with Macquarie Island at 54°S and most sub-Antarctic islands devoid of trees (Van der Putten, et al., 2012b; Hinam and Hope, 2005). The reliance of this technique on subfossil wood recovery from eroding peat deposits further limits its applicability in other areas, where such conditions are unlikely. Preservation of wood remains may also vary between locations owing to differences in peatland ecosystem functioning, for example high decomposition rates would preserve less *Dracophyllum* remains, potentially resulting in the

erroneous interpretation of stronger wind speeds. These factors significantly limit the value of the technique when considering the question of symmetry of wind behavioural changes through time, because it is not applicable across the entire sub-Antarctic region.

3.3.3. Glaciers

Mass balance, the ratio between accumulation of ice and its eventual ablation, determines the extent of glaciers and ice-sheets. Changes in precipitation and temperature are the primary determinants of this balance, and therefore recordings of glacial expansion and retreat made by dating moraine features provide a measure of SHWW variability. The majority of such investigations to date have focused on the South Island of New Zealand as glaciers in the Southern Alps are highly sensitive to changes in SHWW strength and position (Knudson et al., 2011).

Putnam et al. (2010) use ^{10}Be exposure dating to analyse a moraine complex in New Zealand, this moraine complex documents glacier expansion which coincided with the Antarctic Cold Reversal (ACR). It is suggested that the glaciers in the region respond negatively (i.e. retreat) during periods when the SHWW shift southwards because the frequency of cold moist oceanic airflow over the Southern Alps declines under such conditions. Therefore, the expansion of New Zealand's glaciers during the ACR is interpreted as evidence of equatorward shifted westerlies.

The technique has also been applied in southern South America, where precipitation peaks in the core of the westerly winds (~50°S; Fig. 6) and some coastal regions receive up to 7 m yr^{-1}. Precipitation drops rapidly along a

latitudinal transect to the north and south of the core, with values of 2.5 myr^{-1} at 40°S (Hulton et al., 2002). Simplistic glacier modelling studies indicate that the known extent of the Patagonian Ice Sheet during the LGM can only be replicated when lower temperatures are accompanied with precipitation change to a pattern different to present. Precipitation would need to be lower at 50°S and enhanced in the north of the present wind-belt at around 42°S (Hulton et al., 2002). This evidence suggests that at the time of the LGM westerlies shifted by ~10° further north, and hence provides evidence for wind behaviour change over long timescales. Indeed it is now generally accepted from such evidence that the SHWW shift equatorward by ~10° during cold glacial periods and to a more poleward position (~52°S) during inter-glacial periods (Van Daele et al., 2016).

Working in the Chilean Andes, Herman and Brandon (2015) also linked modern precipitation patterns set-up by the SHWW to current glacier coverage. During the LGM the westerly belt and correspondingly peak precipitation were offset by ~600km further toward the equator. Glaciers are powerful agents of erosion, and rates increase as a function of sliding-velocity, itself a product of mass balance. Modelling of glacier erosion rates for a variety of scenarios supports the findings of the earlier work of Hulton et al. (2002).

Within the Southern ocean region there are limited areas where glacial advances can be used as westerly wind proxies. Although most of the sub-Antarctic islands were glaciated during the LGM (Hodgson et al., 2014), the link with the westerlies is not straightforward, and this archive remains unused. One limitation is that although glacial extent can be used for wind behavioural changes over long time-periods the temporal resolution obtainable is very low. This is a result of the

relatively slow response and lag-time of glaciers to external change and difficultly in dating the geological features that are produced with certainty.

3.3.4. Speleothems

Changes in precipitation are also recorded by speleothems which develop in caves, and can be reconstructed using stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) and by measuring their growth rates (Whittaker et al., 2011). Similarly to pollen based climate reconstruction, lake level fluctuations and glacier extent, this method relies on the correlation between modern day precipitation and wind-speed, on land masses with a high axial range (i.e. *New Zealand* (Whittaker et al., 2011) and *southern South America* (Schimpf et al., 2011)). Interpretation follows that speleothem growth rates increase and isotopic ratios are depleted, during wetter periods when westerly airflow is stronger (Whittaker et al., 2011). A specific problem with this interpretation is that the link with precipitation is not direct because speleothems develop as a product of moisture 'drip-rate' into the cave. Therefore, geological alterations of water filtration pathways, or cave ventilation changes, may affect growth rates and isotopic signals independently of climate. Additionally, extensive monitoring is required to understand the isotopic signals from individual cave systems, as a prerequisite of reliable precipitation reconstructions.

One major benefit of the method is the ability to provide precipitation data over long time-periods (~80 k years) whilst maintaining relatively high temporal resolution, owing to slow but continual speleothem growth and precise Uranium-series age-control. Studies of speleothems for the purpose of SHWW reconstruction over the Southern Ocean are currently very rare with only two found for this compilation. They are potentially valuable sources of data, and

exploration of suitable caves on some of the larger sub-Antarctic islands may be a fruitful area of research in the future.

3.3.5. Marine Sediments

Deep Ocean

Climate reconstruction from deep-ocean cores taken inside the westerly wind-belt are currently rare. However, it is important to acknowledge the significant contribution to our knowledge of past SHWW behaviour and subsequently their role in the earth system that has been gained from studying them. This primarily refers to the work of Anderson et al. (2009), who measure concentration of biogenic silica in sediment cores from the Southern Ocean, as a proxy for upwelling. Biogenic silica is produced by marine diatoms that inhabit the euphotic layer of the ocean. The zone of highest present day diatom productivity occurs in the region of maximum deep water upwelling which brings dissolved nutrients, most importantly dissolved Si needed for shell construction to the surface. When the diatoms die their shells settle through the water column and collect in the sea-floor sediments. Therefore, periods of high productivity have stronger deposition, and vice versa. Upwelling of deep-water in the Southern Ocean is principally controlled by the SHWW, and therefore changes in the concentrations of biogenic silica in ocean sediment cores from the region reflect periods of weaker or stronger SHWW influence on the ocean. The same water that is upwelled is also rich in carbon, and the quantity that reaches the surface partly determines the strength of the Southern Ocean carbon sink. Using this proxy Anderson et al. (2009) showed that two separate periods of increased upwelling were both concurrent with increasing deglacial CO_2 levels.

Fjords

Sediments recovered from fjords on the west coasts of southern South America (Lamy et al. 2010) and New Zealand (Knudson et al., 2011) have also been used to extract information on past SHWW conditions. Lamy et al. (2010) use a number of different fjord sites as part of a large multi-proxy and multi-archive palaeoclimate investigation. Specifically they use fjord sediments to provide a record of long-distance wind-transported Andean clays, which provides a wind-proxy that is independent of temperature changes. They also investigate biogenic carbonate accumulation rates, to infer changes in fjord salinity, itself a product of the amount of freshwater input from precipitation. Periods of low salinity occur when precipitation is high, and this reduces marine influence on the system and carbonate production declines. Finally, they measure siliciclastic components and terrestrial organic matter of the sediment, a further record of run-off from precipitation on land.

Knudson et al. (2011) examine sediments from west coast fjords on the South island of New Zealand. As previously described above, the high axial range produces strong orographic rainfall in the west (6-14 m yr^{-1} within the study site) and a rain shadow in the east. Because fjords are semi-enclosed systems, with one or more marine sills and steep walls, circulation within them consists of a low-density brackish surface layer and high-density saline water below. Precipitation, and run-off from the land, controls the contributions of either terrestrial or marine organic matter to the sediments they accumulate. For example, during high precipitation periods, freshwater input increases the stratification of the fjords and allows the surface low-salinity layer to outcrop further seaward. Therefore, more terrestrial organic matter (carried from run-off water) will enter the fjord sediments, and will also be carried further seaward

before it is deposited. Relative changes in terrestrial and marine organic matter in sediment cores can be determined by measuring the C:N ratio and δ^{13}C. High C:N ratios and depleted δ^{13}C indicate terrestrial organic matter, and vice versa. Since terrestrial plants are cellulose and carbon rich when compared to marine algae. An advantage of this technique is that it provides a simple proxy for wind driven rainfall, affected less by other variables such as temperature which is a problem faced by lake-level and glaciological methods (described above). Nevertheless, other disadvantages associated with wind reconstruction using precipitation (discussed above), such as the assumption that wind-precipitation correlations remain constant through time also limit this technique.

3.3.6. Concluding Remarks

Westerly wind reconstructions from the Southern Ocean, and consequently our understanding of wind variability through time, face a number of limitations that are described in this review. The primary limitation for existing palaeowind studies is that many records rely on convoluted indirect proxies, which require multiple assumptions to link a measured environmental variable (e.g. precipitation) with wind conditions. Each level of separation between the environmental indicator and wind behaviour introduces more uncertainty into the reconstruction. Unfortunately, pure wind proxies i.e. those where a measureable indicator responds directly to wind behaviour, remain elusive and under-investigated despite their potential to yield reconstructions with greater reliability.

Current knowledge is significantly reliant on palaeohyrological studies, including; lake-level changes, speleothem growth rates, ecotone migrations, fire regimes, and the extent of glaciers. These studies are especially concentrated in southern South America (Fig. 5; Table. 4), where the present day wind-precipitation

correlation is strong. However, the potential divergence of wind and precipitation correlation through time is an insufficiently quantified source of uncertainty that challenges this technique. Also each palaeohyrological proxy records slightly different moisture data (Kohfeld et al., 2013), and therefore datasets may not be mutually compatible when used as part of a large dataset to compare model outputs. As a result, although these records are useful, they must be interpreted with caution.

Aeolian transport proxies, such as dust and foreign pollen, provide a more direct link to wind behaviour. However, they are currently rare and their relationship with wind strength is not necessarily straightforward. Nevertheless, the method is potentially useful and warrants further research. Although they are direct, exposure based proxies (e.g. Tree line altitude) require specific vegetation and provide only low temporal resolution records. It is therefore likely that their future application will be limited.

High-resolution chronologies are needed to determine how wind behaviour changed during short-climate intervals, but the responsiveness of the proxy to these events is also crucial. Reconstructing how the winds responded to short climate intervals has potential to yield important information for calibrating models under different climatic conditions. Of the palaeowind studies, which have been conducted in the Southern Ocean region, peatlands and lakes are the most frequently utilised archives (Fig. 5). They are considered advantageous in that they incorporate an abundance of easily dateable material and can therefore by used to develop the high-resolution reconstructions of westerly wind change needed to study Holocene and late-glacial variability.

The concentration and increasing number of SHWW reconstructions from southern South America is positive, although tight clustering of records is also problematic in introducing bias when extrapolating wind changes across the whole Southern Ocean region. It is assumed this trend is due to the accessibility of the region, and therefore continued interest is expected. Lack of data further south limits our ability to accurately isolate the signals of latitudinal shifts, expansion and contraction, and general weakening or strengthening of the winds (Kohfeld et al., 2013) or to fully assess the hemispheric symmetry of such changes (Fletcher and Moreno, 2011, 2012). There remain no systematic studies covering the whole Southern Ocean region, despite the presence of numerous island groups in the sub-Antarctic with suitable palaeoclimate archives covering the Holocene period (*see section 3.1*). The sub-Antarctic islands lie within the core belt region, and therefore are ideally situated to study wind behaviour. There are however no available methods that are applicable to all of these potential sites. This study presents a novel, direct wind proxy that is potentially applicable to the entire sub-Antarctic.

3.4. Methodological Rationale

Despite the potential of sub-Antarctic peatlands as palaeowind archives, they remain understudied since few proxies that can be used to infer changes from the record are currently available. Given the availability of these archives and numerous calls to improve our understanding of past westerly wind wind behaviour, it is surprising that such a significant data gap still exists. In response to the review of current wind-reconstructions presented above, this research aims to develop a novel peatland based palaeowind proxy that is; (1) directly linked to

wind-conditions, (2) applicable throughout the sub-Antarctic region, and (3) simplistic to allow efficient field sampling.

Deposition of oceanic base cations (salts) onto the terrestrial wetlands (peatlands and lakes) is established as a basis for wind-behaviour reconstruction (*as outlined above:* Saunders et al., 2009, 2015). During periods of

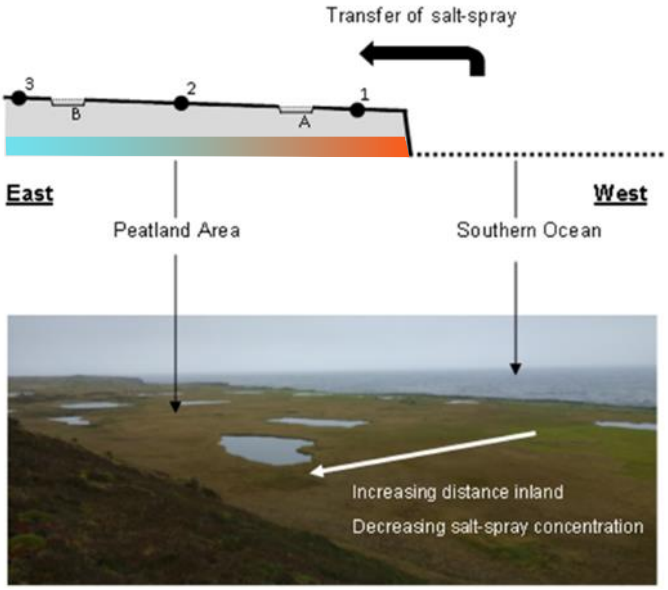

Figure 7 | **Schematic illustration demonstrating the utilisation of variable oceanic base cation inputs to coastal peatlands as a palaeowind proxy.** Photograph demonstrates the suitability of low-elevation coastal peatlands on the sub-Antarctic islands as palaeoenvironmental archives, due to their limited mirco-topographic variability and proximity to the Southern ocean (sea-spray source). Colour bar indicates concentration of salt-spray input (red-high; blue-low). Numbers 1-3 refer to peat core and surface samples locations and A-B highlight that lakes (lacustrine sediments) are also present within the salinity gradient. Image source: Dominic Hodgson.

stronger winds the terrestrial salinity increases (and vice versa) so reconstruction of surface salinity conditions relates directly to changes in wind strength (Fig. 7). Base cation deposition has already been shown to measurably alter the way that peatland ecosystems function, for example the type of plants that colonise the surface (Whittle and Gallego-Sala, 2016; Kleinebecker et al., 2008), and these changes are recorded in the peat sequence as it develops. In addition, testate amoebae have been recorded in salt influenced soils (e.g. Barnett et al., 2016)

and previous studies of their distribution in the sub-Antarctic commonly cite measures related to salinity (e.g. conductivity and pH) (Table. 5) as community structuring variables. However, explicitly testing this relationship has not been undertaken before, and only limited data on the ecological preferences of testate amoebae in the sub-Antarctic region is available more generally (Table. 5).

Therefore, the proposed method is to link, via a statistical transfer function, the ecological amplitudes of peatland testate amoebae species to concentrations of (oceanic) salts deposited in their habitat. This is achieved by quantifying assemblage changes along the present day surface conductivity gradient on sub-Antarctic Marion Island.

Table 5 | Summary of recently published testate amoebae-environment responses to continuous variable the Southern Ocean region. n = number of surface samples used in the study.

Location	Sample type	n	Community structuring variables identified	References
Île de la Possession	Aquatic	66	Conductivity, pH, NH_4^+	Vincke et al., (2004b)
	Terrestrial	89	pH, $CaCO_3$, moisture, chloride, PO_4^{3-}	Vincke et al. (2004a)
	Terrestrial (Ornithogenic)	79	pH, moisture, conductivity, NH_4^+, PO_4^{3-}	Vincke et al. (2007)
South Georgia	Aquatic	45	pH	Beyens et al. (1995)
	Terrestrial / Aquatic	58	Moss type, moisture	Vincke et al. (2006)

The new methodological approach has the potential to independently generate wind-speed reconstructions to validate results of diatom and plant-macrofossil based salt-spray proxies currently in development at the British Antarctic Survey (Saunders et al., 2015). The wider intention of this research project is to develop a number of complimentary SHWW proxies applicable across the whole Southern Ocean region and capable of providing improved boundary conditions for environmental modelling studies.

The new proxy proposed in this thesis can be considered analogous to the recent developments of the use of foreign pollen influxes to sub-Antarctic peatlands (i.e. it is based on the reconstruction of foreign material (base cations) from an upwind source region). However, the notable advantage of this technique is that the ocean provides a stable and unlimited supply of ions, compared to dust and pollen proxies where changes in the source region may affect the proxy interpretation.

Advantages of proposed method:

1. Peatlands are ubiquitous archives throughout the Southern Oceans islands (*see* section 3.1), meaning that the method can be applicable to the whole region, allowing standardisation of methodology.
2. Peatlands preserve more than one potential biological proxy (e.g. macrofossils and diatoms) which can provide a more in-depth picture when considered together, and allow independent testing of each individual proxy.
3. Peatland sequences can be dated relatively easily. There is an abundance of organic material, so a robust chronology can be developed with radiocarbon dating. Other dating techniques such as tephra analysis and ^{210}Pb can also be applied. Accumulation rates are high enough to produce a high-resolution record spanning the Holocene period (Table. 2).
4. The westerly winds have a direct effect on the coastal peatlands ecosystems. There is no requirement for assumptions of continual correlation between the winds and other environmental conditions, which is problematic for other proxies used currently.
5. Peatlands on the sub-Antarctic islands are interspersed with lakes. Testate amoebae and diatoms are present within both archives meaning

that records can be directly compared, allowing a unique multi-archive inter-comparison.

Chapter 4: Study Site

4.1. Modern Climate and Geographical detail

The Prince Edward Island group form part of the Southern Indian Ocean province of the sub-Antarctic (Van der Putten et al., 2012a) and includes Marion Island (46°55'S, 37°45'E; *see* Fig. 1) and its smaller neighbour (namesake of the group).

Marion Island, which is the subject of this study, has a land area of 293 km^2 ; 19 km long, 12 km wide, and a broadly conical shape rising to central highlands that peak at 1,230 m above sea-level. It is uninhabited except for an operating research base of the South African National Antarctic Programme (SANAP) on the eastern coast (~20 m above sea level). Meteorological observations have been recorded from this location since 1949, which provides one of the most complete datasets available for the sub-Antarctic (Hedding et al., 2015; Smith, 2002).

Climate conditions are hyperoceanic (Hedding et al., 2015); cold, wet and with constant westerly wind. Mean yearly temperature is 6.31°C (period 2010-15) and consequently remnant permafrost is sporadic and isolated to upland areas >1,000 m above sea level (Boelhouwers et al., 2003 *and references within*). Previously, researchers have commented on the exceptional thermal stability; diurnal (1.9°C) and seasonal (3.6°C warmest – coldest month mean) (Smith, 2002; Yeloff et al., 2007), although more recent data suggests that this may be increasing to ~5°C (Fig. 8).

Precipitation is high, ~1,400 mm (2010-15), and is mostly delivered as rain (Smith, 2002). Orographic effects are likely and therefore west coast precipitation may be significantly greater. Average surface airflow is 8.4 ms^{-1} (daily; 5 m height) and gusts up to 55 ms^{-1} have been recorded. Gales exceeding 18.3 ms^{-1} occur >100 times annually and each last up to 10 hours (Hedding et al., 2015). No specific data exists for airflow velocities on the west coast of the island, although higher values are expected due to greater exposure to the prevailing westerlies. Periods of high airflow velocity are associated with precipitation bearing weather fronts (Hedding et al., 2015) and combined with orographic influence of the islands western slope, it is likely that wind-speed significantly correlates with precipitation. Unfortunately, to the author's knowledge, data is not yet available to confirm this interpretation.

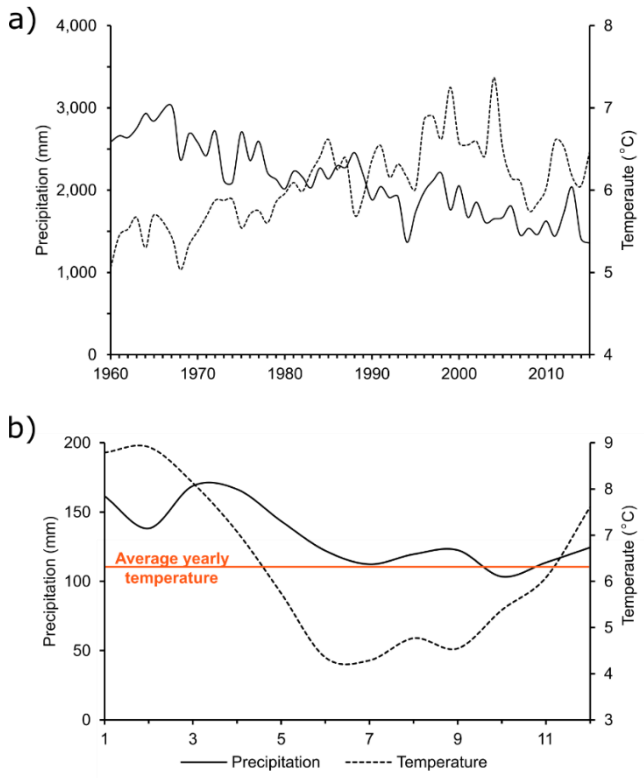

Figure 8 | Marion Island climate data from the SANAP weather station on the east coast (NCDC, 2016)**.** a) 1960-2015 average values of yearly precipitation and temperature, b) seasonal climate variability calculated from monthly means for the period 2010-2015. Numbers indicate month of the year (January 1 – December 12).

4.2. Geology and environment evolution

The island is a geologically-young (0.45 Ma) active intraplate volcano (Yeloff et al., 2007; Hodgson et al., 2014) on a small submarine plateau, and has never been attached to another landmass or continent (Van de Vijver et al., 2008). Since formation the landscape has been shaped by episodic periods of volcanism (8) and glaciations (5), as determined by K-Ar dating (McDougall et al., 2001). Pleistocene volcanic activity is evident from grey-lavas at higher elevation which have been modified glacially into moraines and tills (Hedding et al., 2015). A distinct black-lava (aa and pahoe flows) of Holocene age covers much of the remaining surface (Global volcanism program, 2013). Radiocarbon dated peat deposits, vegetation cover and lack of glacial imprint imply that these flows date from between 15-4 k yr. B.P., although lack of vegetation in some areas imply that others may be more recent (Verwoerd et al., 1981). The distinctive (130) scoria cones which are distributed across the surface are also thought to date from Holocene volcanism. Volcanism is not suggested to have caused extinction of vegetation from the island (Yeloff et al., 2007).

During the LGM the island was covered by an ice-mass with glaciers that extended beyond the current coastline onto the continental shelf (Hall et al., 2010; Hodgson et al., 2014). However, the presence of developed periglacial landforms (patterned ground and solifluction terraces) show inland nunataks were present during this period and evidence suggests that these areas acted as biotic refugia (Hodgson et al., 2014). Raised beaches and palaeocliffs are present around the islands periphery and suggest either the island is experiencing tectonic uplift and/or isostatic rebound (Hodgson et al., 2014).

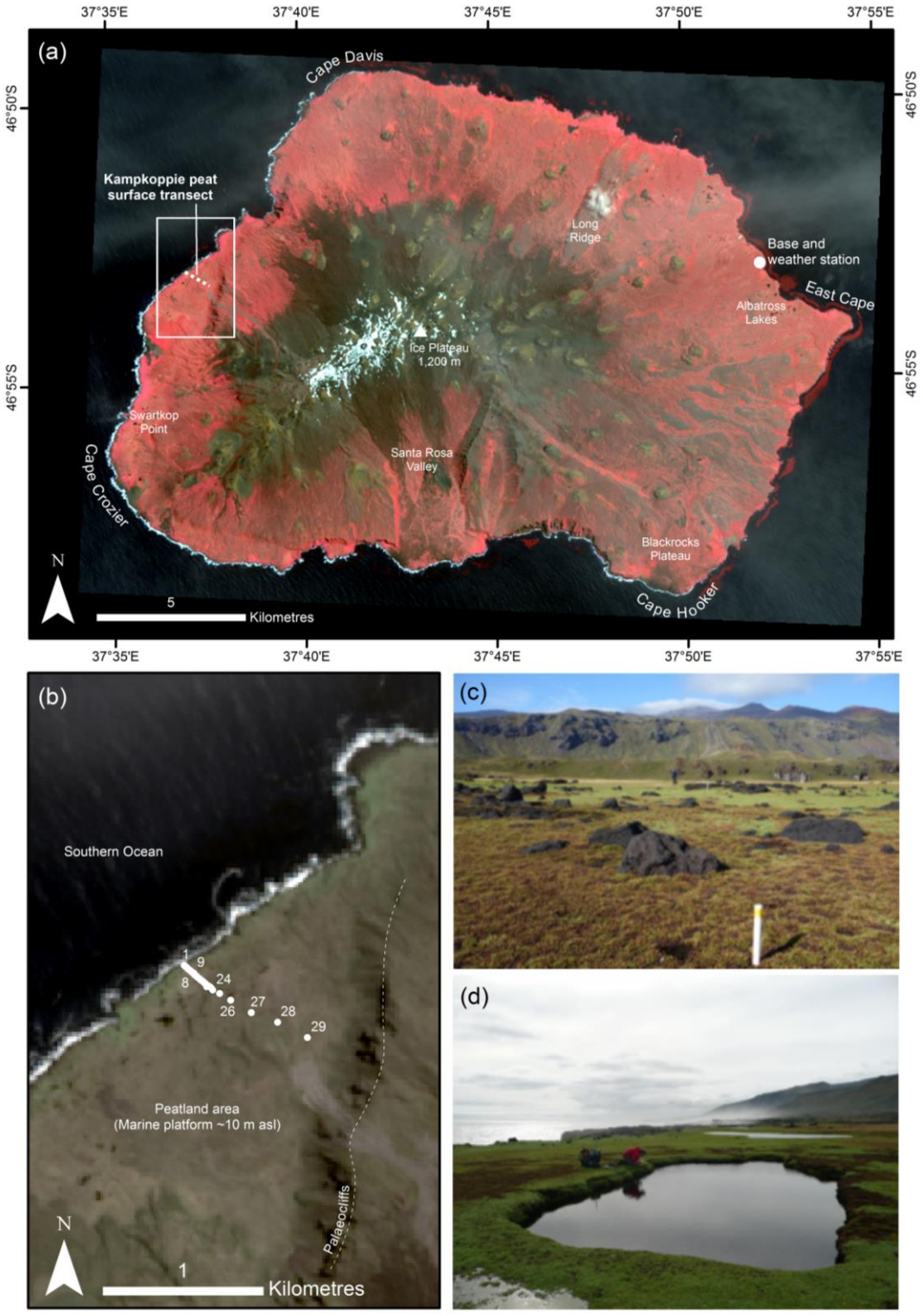

Figure 9 | Marion Island and the peatland surface transect at Kampkoppie. (a) Colour infared Landsat-8 image showing the flat raised marine platform, palaeocliffs in the Kampkoppie area, and general topography of the island including the numerous scoria and cinder cones. Image acquired during the austral summer (01/02/2016) shows growing vegetation (red) and illustrates the extensive coverage of accumulating peatlands in the coastal lowland areas. Higher elevation and more exposed areas (fellfields) have a limited vegetation cover. Band combination; RGB 543. Path 160, Row 93. Aerial mage courtesy of the U.S. Geological Survey. Downloaded from http://earthexplorer.usgs.gov/. (b) Land-sea transect at Kampkoppie (used for this research). Aerial image courtesy of the U.S. Geological Survey. Downloaded from http://earthexplorer.usgs.gov/. (c) Photograph of the transect taken from the coast looking inland toward the palaeocliff. Source: Dominic Hodgson. (c) Pool on the peatland surface with visible salt spray/sea fog in the background. Photograph taken looking northwards along the coast toward Cape Davis. Source: Elie Verlyen, Wim Van Nieuwenhuyze.

The timing of the last deglaciation marks the period covered by the peatland record, since glacial climates and limited ice-free areas would prevent wide-spread peat accumulation. The exact timing of de-glaciation is uncertain because the glacial landforms are either insufficiently dated or have been over-printed by Holocene lava flows. Van Der Putten et al. (2010) extrapolate radiocarbon dating to the base of a 3 m peat sequence from Albatross ridge which indicates that peat initiation had begun by 17,320 yr B.P. It is possible therefore that either; de-glaciation began earlier than expected, the peatland formed in an ice-free refugia, or the dating is erroneous. The latter is most likely, although there may also be merit in the refugia hypothesis.

Other peat initiation dates on the island are much younger (Table. 2) but these are problematic because they may constrain dates of Holocene volcanism and not deglaciation. A compilation of 8 peat cores (Table. 2) shows that average peat depth is 2.9 m, and the average initiation time was ~9.1 k yr B.P. During deglaciation ice receded to a small cap on the islands peak, and had rapidly disappeared due to climate change and/or geothermal heating (Hodgson et al., 2014) by the 1990s. Although glacial geomorphology and volcanism are the most powerful factors shaping the landscape of Marion Island, Aeolian processes are also important due to constant strong westerly airflow. For example, Megaripples formed by the removal of sand-sized particles were observed by Hedding et al. (2015).

In 1980 (February-October) a volcanic eruption at Kaalkoppie on the west coast (Fig. 9) produced two new cinder cones, three black lava flows and deposited a red-black tephra. Although the event was not registered at the research base in

the east, several centimetres of ash were deposited locally (over 2 ha) from the epicentre. Lava flows emanated from an east-west fissure 5 km in length from the Kaalkoppie summit to the coast (Global volcanism program, 2013). Combined they covered a total of 7 hectares, and produced a new coastal front. Another eruption occurred in 2004, but again was minor and affected only a localised area in the South of the island.

4.3. Modern Vegetation

The vegetation of Marion island generally reflects its isolation with a low number of species; 23 vascular plants, 94 mosses, and 47 liverworts (Van de Vijver et al., 2008). Peatlands are abundant in the lowland areas, and cover approximately 2260.5 ha (Yeloff et al., 2007; Van de Putten et al., 2012a; Smith et al., 2001).

Smith & Steenkamp (2001) developed a habitat classification for Marion Island which identified 6 distinct peatland (mire) habitats. All are physiognomically similar and share common vegetation and soil characteristics (Smith et al., 2001). Peat forming plant communities are graminoid rich (e.g. *Agrostis magellanica*, *Uncinia compacta*, *Juncus scheuchzerioides*) and bryophytes are co-dominant. The peatlands are flat, without a microtopography of mounds and hollows (Yeloff et al., 2007). Van der Putten et al. (2012a) indicate that peatland communities are found on valley floors, however they are also present in exposed coastal settings (Fig. 9).

Coastal peatlands are dominated by species such as *Crassula moschata*, *Agrostis magellanica* and *Ranunculus biternatus*. Across the island they are uncommon (~13.6 ha total extent) and found only where drainage is impeded and the substrate is a compacted peat subjected to high concentrations of salt-spray input (mean pH= 4.9) (Smith et al. 2001). Sea-spray complexes occur at the cliff

edges where salt concentrations are very high and are dominated by *Cotula plumose* and *Crassula moschata.*

Where peatlands do not occur, areas with better drainage are covered by fernbrake vegetation, and fellfield complexes dominate higher elevation and more exposed areas. Much of the island is bare rock, with lichens and occasional cushion-forming dicots (e.g. *Azorella selago* and *Colobanthus kerguelensis*) (Yeloff et al., 2007). The bathymetry of the near coast sea-floor is sufficiently shallow to allow the development of kelp beds around much of the island (Fig. 9) and has potential to be used to reconstruct past glaciation (Hodgson et al., 2014). The island has no native grazing macro-herbivores, and insects play only a minor role. Yeloff et al. (2007) show that, during the past 5.5 k yr., deposition of tephra has been a major driver of long-term ecosystem change, that periodically resets succession.

4.4. Recent climate change

During the past 40 years the climate of Marion Island has changed substantially. Precipitation has declined by 45% since 1960, when the (decadal) yearly average was 2726 mm compared to 1878 mm in the 2000s. The trend has continued with 1671.4 mm recorded in 2011 (Smith, 2002; Hedding et al., 2015). Surface air temperature (yearly averaged) has risen sharply by 1.2°C between 1969 and 1999 (Smith, 2002). It is thought that warming is associated to recent increases in sea-surface temperature (Smith, 2002). Over a similar period (1951-1999) average total sunshine hours has risen by 3.3 hours yr^{-1}. Additionally salt-spray input concentrations may be changing due to alterations of atmospheric circulation patterns and wind intensity (Yeloff et al., 2007). These changes are

similar to those observed across the sub-Antarctic including Macquarie, Kerguelen and Heard Island (Smith, 2002).

Increasing air temperature and declining precipitation are expected to increase the rate of microbial decomposition in Marion Island's peatlands, especially considering they are currently more strongly limited by waterlogging than temperature. Smith (2002) suggest that decomposition will provide more nutrient for primary production, which will also benefit from warmer temperatures, although this is currently untested.

Impacts of direct anthropogenic disturbance are minimal on the island. Human visitation has only been occurring in the last two centuries, therefore natural archives do not preserve a record of long-term human influence (Yeloff et al., 2007). Early visitors to the island introduced a variety of alien species, which have since been eradicated.

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Chapter 5

Testate amoebae of salt-spray influenced peatlands on Marion Island and their potential for reconstructing Late Quaternary changes in the Southern Hemisphere Westerly Winds.

Abstract:

Sub-Antarctic peatland ecosystems provide rare terrestrial archives of past environmental conditions over the Southern Ocean. Peatlands located on exposed western coasts receive aerosol inputs of oceanic base cations in concentrations proportional to the intensity of prevailing westerly winds. Reconstruction of bog-surface salinity through the peat record could provide a direct proxy for past changes in wind behaviour, however methods suitable to extract this signal are lacking. We propose and test the idea that testate amoebae can record bog-surface salinity changes. Using a land-sea transect on Marion Island (sub-Antarctica) we examine the ecology of these microorganisms and test their response to the contemporary conductivity gradient. From 25 surface samples we recorded; 35 taxa of 18 genera, and twelve taxa not previously documented on Marion Island. Conductivity explained the most variance in the community data. For the first time we quantify ecological optima and tolerance of testate amoebae to conductivity conditions, and develop an exploratory statistical transfer function. We find that conditions rapidly become unsuitable for colonisation by most testate amoebae taxa after an apparent threshold level of high salinity is reached. The initial results demonstrate the potential of our novel proxy for palaeowind reconstructions throughout the wider sub-Antarctic region.

Introduction:

Testate amoebae are a polyphyletic group of organisms that are abundant on the surface of ombrotrophic and minerotrophic peatlands[1–3], and also inhabit other

freshwater ecosystems around the world[4,5]. Assemblages of these organisms reflect dominant environmental conditions, including moisture, pH and temperature. They form decay resistant tests which remain identifiable to a low taxonomic level in sedimentary sequences, allowing assemblage change through the Late Quaternary to be studied[6]. Populations can double approximately 10-27 times per year[1], and so they react rapidly and sensitively to changing environmental conditions[1]. Consequently, in peatlands, they record environmental changes at a resolution limited only by the rate that organic matter accumulates. For these reasons, the group have been used widely for high-resolution inferential evidence of past climate change[7,8], and to a lesser extent, for monitoring land-use change and ecosystem restoration[9]. Another well-developed use exploits the response of saltmarsh testate amoebae assemblages to salt water inundation frequency, as a proxy for past sea-level change[10–13].

The sub-Antarctic islands are a dispersed collection of small landmasses in the Southern Ocean, located within 10-15° latitude of the Antarctic Polar Front (total land area approximately 26,000 km^2)[14]. They are strongly influenced by the Southern Hemisphere Westerly Winds (SHWW), due to their location in the core wind belt (50-60°S)[15]. As the strongest time-averaged oceanic winds in the world[16], the SHWW effect large-scale circulation of the Southern Ocean in a way which regulates upwelling of carbon rich deep-water and consequently influences ocean-atmosphere CO_2 balance. It has been proposed that this mechanism is responsible for governing glacial-interglacial atmospheric CO_2 levels[16–18]. Changes in the SHWW also play an important role in regulating precipitation supply throughout the Southern Hemisphere[19,20] as well as ice-sheet contributions to global sea-level[21]. It is therefore significant that recent observations show an intensification (+6% strength) and poleward migration (~2°S summer, ~3.5°S winter) of the wind-belt in recent decades (1981-90 relative to 2001-10[22]) associated with greenhouse-gas induced climate warming and ozone depletion[23,24]. Demand for accurate reconstructions of wind-behavioural change since the Last Glacial Maximum has increased, because they are needed to inform the models used to predict future changes.

Providing suitable reconstructions from high-resolution terrestrial archives is challenging to palaeoenvironmental research for two major reasons: (1) the

Southern Hemisphere is characterised by a limited land-area, and (2) linking wind-behaviour to an environmental variable that can be reconstructed over long time-periods often requires many significant assumptions (e.g. attempts to use dust deposition[25], pollen[26], and glacier extent[27,28]). Consequently, most existing records originate from the continents surrounding the Southern Ocean, outside of the core wind-belt, and use precipitation and rainfall as wind-proxies. The exception are a small number of palaeoclimate sites in South America which are frequently used as it is the only landmass to intersect the current belt of most intense westerly winds[26,29,30]. Equally, most proxies are highly convoluted so they are hard to compare and reliability is often questionable. Combined it is unsurprising that palaeo-data is often contradictory between records and models, even at local-scales.

The sub-Antarctic islands therefore represent rare and understudied opportunities to develop high-resolution terrestrial-based reconstructions from inside the core SHWW belt. However, attempts to apply traditional palaeoecological techniques have returned limited success[29,31], and the existing wind-behaviour proxies (e.g. foreign pollen influx[32,33] and tree-line altitude[34]) are not applicable to all available islands.

The sub-Antarctic islands are characterised by strong west-to-east salinity gradients, set-up by deposition of sea-spray from sustained westerly airflow (mean wind speed is 6-15 ms^{-1})[14]. Salinity increases during periods of stronger wind as higher concentrations of oceanic base cations are brought to the land by the westerly winds. Because they have been used previously in salt-marshes, we propose that testate amoebae in coastal peatlands respond significantly to changes of this gradient. The main aim of this paper is to test the link between the distribution of testate amoebae and contemporary inputs of salt-spray to a coastal peatland on Marion Island. We test this response at the lowest possible taxonomic level (i.e. optima-tolerance of individual taxa) and at the community level (i.e. spatial heterogeneity in testate amoebae activity and biomass).

A secondary aim of the paper is to contribute to the increasing knowledge of sub-Antarctic testate amoebae diversity, which remains poorly known, especially compared to similar latitudes in the Northern Hemisphere. It has been proposed that diversity broadly decreases poleward, through the sub- and maritime-Antarctic regions[35]. If this trend holds, the diversity currently reported for Marion

Island must be underestimated, as a higher diversity would be expected for its latitudinal position.

Remote islands, such as in the sub-Antarctic, are key sites to investigate testate amoebae biogeography and possibly to identify new species with a limited geographical distribution. The third and final aim of the paper is to contribute to the discussion on whether *Argynnia antarctica* constitutes a species which is endemic to Marion Island[36], a claim that has been put forward despite limited morphological evidence to support it[37]. A more recent study[37] used samples from Île Amsterdam to demonstrate that an overlap in the dimensions exists between *A.antarctica* and *A.dentistoma* individuals, suggesting these are not two separate species. Our dataset will further inform this discussion through contribution of a vastly improved quantity of measurements of individuals from the Marion Island population.

Study Site:

Marion Island (46°55'S, 37°45'E) is an extremely isolated 293 km^2 landmass in the South Indian Ocean province of the sub-Antarctic biome[38]. It is conical in shape with a central active volcanic peak (1,230 m a.s.l.) and extensive coastal lowland plateaux punctuated by scoria cones. The island formed approximately 0.45 Ma ago, and has never been attached to another landmass or continent[39]. The modern landscape is a product of a complex volcanic and glacial history[14].

The climate is hyperoceanic[40]; cold, wet and dominated by constant westerly airflow. Mean yearly temperature is 6.31°C (2010-15), and seasonally stable (3.6°C warmest – coldest month)[41,42]. Mean annual precipitation is (1400 mm; 2010-15) and mostly delivered as rain[41]. Gales, with winds exceeding 18.3 ms^{-1} occur >100 times annually and each last up to 10 hours[40]. Remnant permafrost from the Last Glacial Maximum is sporadic, and isolated to areas >1,000 m a.s.l. There is no longer any permanent ice-cover.

Vegetation composition reflects the isolation, with a small number of species; 23 vascular plants, 94 mosses, and 47 liverworts[39]. Peatlands are abundant in the lowland areas, and cover approximately 2,260 ha[38,39,42]. Peat forming communities do not include the *Sphagnum* spp. which dominate Northern peatlands, instead they are graminoid rich (e.g. *Agrostis magellanica, Uncinia compacta, Juncus scheuchzerioides*) with some bryophytes also present. The

peatlands are flat, without a microtopography of mounds and hollows[42]. Direct anthropogenic disturbances of the peatlands are minimal, since human visitation of the islands has only occurred in the last two centuries, although a number of invasive species have been introduced[41].

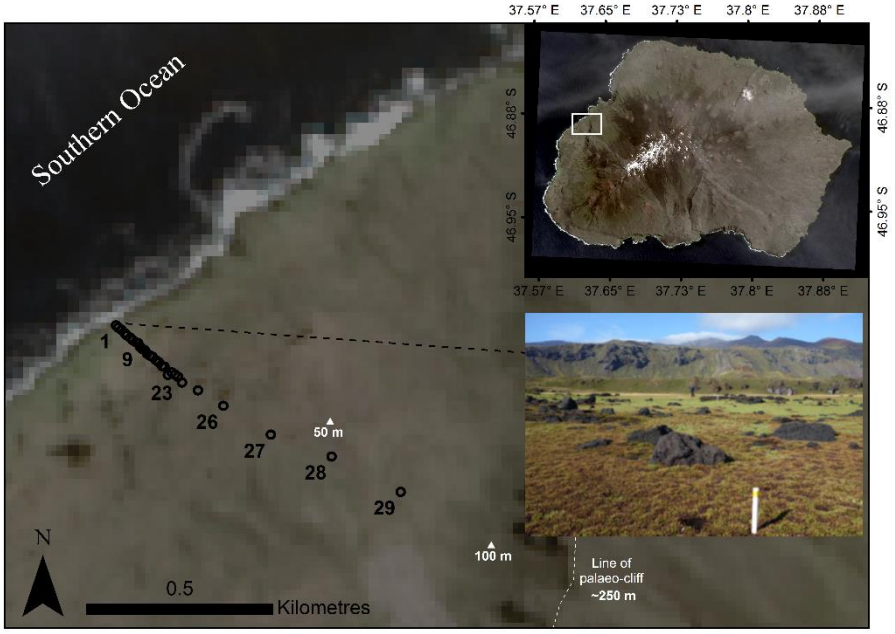

Figure 1 | The low elevation coastal peatland at Kampkoppie, western Marion Island (inset)**.** Markers indicate the positions of sample stations (1-29) along the land-sea transect. Photograph depicts land-cover and topography of the peatland surface, looking directly inland from sample station 1. Spot heights in metres above sea level are indicated (white triangles). Landsat natural colour image downloaded from (http://earthexplorer.usgs.gov/). Photograph source: Dominic Hodgson.

Methods:

Field methodologies

The samples used in this report were collected during a field campaign in April-May 2013. A single linear transect was established across an area of low elevation coastal peatland at Kampkoppie, on the west-northwest coast of the island (Fig.1). The transect spans a coastal plateau separated from the sea by a low cliff, so altitudinal range was <50 m (Fig.1; Fig.S1). The transect stretched 800 m inland from the coast, and was aligned with the direction of the prevailing westerly winds (i.e. west-east). The position and layout was chosen to avoid rock intrusions and macro-topographic hollows and ponds in the peatland surface.

Twenty-nine sampling stations were selected at intervals inland from the coast (1=coast, 29=inland); stations 1-24 were spaced every 10 m and 25-29 every 50-150 m. The location of each sample station was recorded with a handheld GPS.

At each station, surface peat monoliths approximately 500 cm^3 (10x10x5 cm) were sampled using a trowel, and placed into re-sealable plastic bags. Samples were collected on the same day to mitigate potential effects of weather variability. All samples were frozen for transport and storage at the British Antarctic Survey prior to laboratory analysis. The vascular plant species and occurrence of bryophytes at each station were recorded. No in-situ measurements of water chemistry or water table depth were obtained.

Laboratory methodologies:

Monolith cores were sub-sampled whilst frozen using a band-saw. To isolate and concentrate testate amoebae for analysis, 1-cm^3 of surface vegetation litter from each monolith was measured via displacement in distilled water. Samples were prepared following the protocol proposed by Charman et al. (2000)[4], a method widely accepted for concentrating tests from a diverse range of environments globally[43,44]. The organic litter was boiled in deionised water for ten minutes to disaggregate the testate amoebae, in preference to soaking for twelve-hours[43]. The material was sieved and the fraction >15 µm <300 µm retained. These residues, containing the concentrated testate amoebae, were stored in deionised water prior to counting.

Semi-permanent microscope slides were prepared by diluting residue with glycerol[2]. Glycerol dispersed the sample to aid identification, permitted multidirectional viewing of tests and gave each slide a longer lifetime. Residues were thoroughly mixed before producing slides to counter preferential settling of different fractions of test size. Counts, species identification and test measurement were completed at x400 magnification. Identifying characteristics used to distinguish individual species included; shell composition, shape, ornamentation, size and colour[3].

Minimum counts of 100 tests within each sample were set as the threshold for a sample to be included in subsequent analysis. In three samples, counts >25 tests were not possible and so these samples were removed. Morphological identifications were based on: Charman et al. (2000)[4], Ogden and Hedley (1980)[45] and published photographs of taxa not included in these guides[3,37]. We also used supplementary photographs of Southern Hemisphere specific[46–48], and typical salt-marsh species[49].

A Zeiss AxioImager A1 light microscope, with an AxioCamHR3 coupled to AxioVision software version 4.8.2.0, was used to identify, count and measure tests. For morphological analysis we recorded measurements of test width, length and width of aperture. Additionally, for species with a hooded aperture (e.g. *Corythion dubium* and *Centropyxis* spp.) we measured aperture length, and for the species *Apodera vas,* we measured neck length as a distinguishing characteristic[47] (Fig.S2).

Concentrations of tests were calculated from parallel counts of a known quantity of exotic pollen spores, added at the disaggregation stage of preparation[43]. Bulk density was used to convert concentration by volume into tests per gram of dry mass[44]. Biovolume estimates were calculated per species, using equations for different categories of geometric test shapes by Mitchell (2004)[50]. Average test dimensions, for each species, were calculated using measurements from the Marion Island population, and other records from published literature (Table.S1). The factor 1 μm^3=1.1x10^{-7} µg C was used to convert shell volumes to carbon biomass[50].

The Shannon-Wiener diversity index (SWDI) for testate amoebae data was calculated for each sample using the equation:

$$SWDI = -\sum_{i=1}^{S}\left(\frac{ni}{N}\right) \times ln\left(\frac{ni}{N}\right)$$

Where *ni* is the number of individuals of one species in a sample, *N* is the total abundance of organisms and *S* species richness of each sample[3,44]. Resultant values indicate the relative health of the environment; healthy 2.5-3.5, in transition 1.5-2.5 and stressed 0.1-0.5[3]

Environmental information for each sample site was provided by extracting pore water from the peat monoliths. We thawed each sample and collected melt water, and also applied a vacuum to remove further pore water for analysis. Water samples were dispensed into acid-washed bottles and frozen awaiting processing. We tested variability in water conductivity (salinity) with distance inland from the coast, using a calibrated Hanna probe (HI 98129) which compensated values to a standard temperature. Under the laboratory conditions (~20°C and 1 atm pressure), conductivity is linearly related to salinity (R^2=0.998) and therefore provides a good proxy for concentrations of oceanic salts[51].

Recordings of pH were also made using the same probe. A sub-sample from the surface, 2-cm^3 in volume, was removed for bulk density and moisture content analysis. Bulk density was calculated by dividing sample mass after oven-drying to constant mass by sample volume[52]. Moisture content (%) was calculated from the decrease in sample weight after drying[52].

Statistical methodology:

Testate amoebae counts were converted to relative abundance (%) prior to analysis, and samples with counts < 100 were removed from the dataset. Detrended correspondence analysis (DCA) without transformation or down-weighting of rare species was used to check the gradient length of the first DCA axis to determine whether species distribution was unimodal or linear. Gradient length was more than 3 (DCA1 = 3.8) and therefore unimodal ordination techniques were used for subsequent analysis. Relationships between the testate amoebae communities and environmental variables (conductivity, pH, moisture content, bulk density, distance to coast) was investigated using canonical correspondence analysis (CCA) ordination in CANOCO v4.5[53]. The significance of each environmental variable of the testate amoebae assemblages was tested by performing individual CCA for each variable as the only explanatory environmental variable.

Species ecological optima and tolerances were determined using simple weighted averaging. Transfer functions for predicting bog-surface conductivity were built from assemblage-based training sets using regression modelling in R[54]. Transfer functions were constructed using several regression models; weighted averaging (WA), tolerance-downweighted weighted averaging (WA-tol), weighted averaging partial least-squares (WA-PLS) and maximum likelihood (ML). Model performance was assessed using r^2 and root mean square error prediction (RMSEP) with leave-one-out cross validation. Model performance was improved when 8 samples with residuals exceeding 20% of the standard deviation in the observed conductivity values were removed.

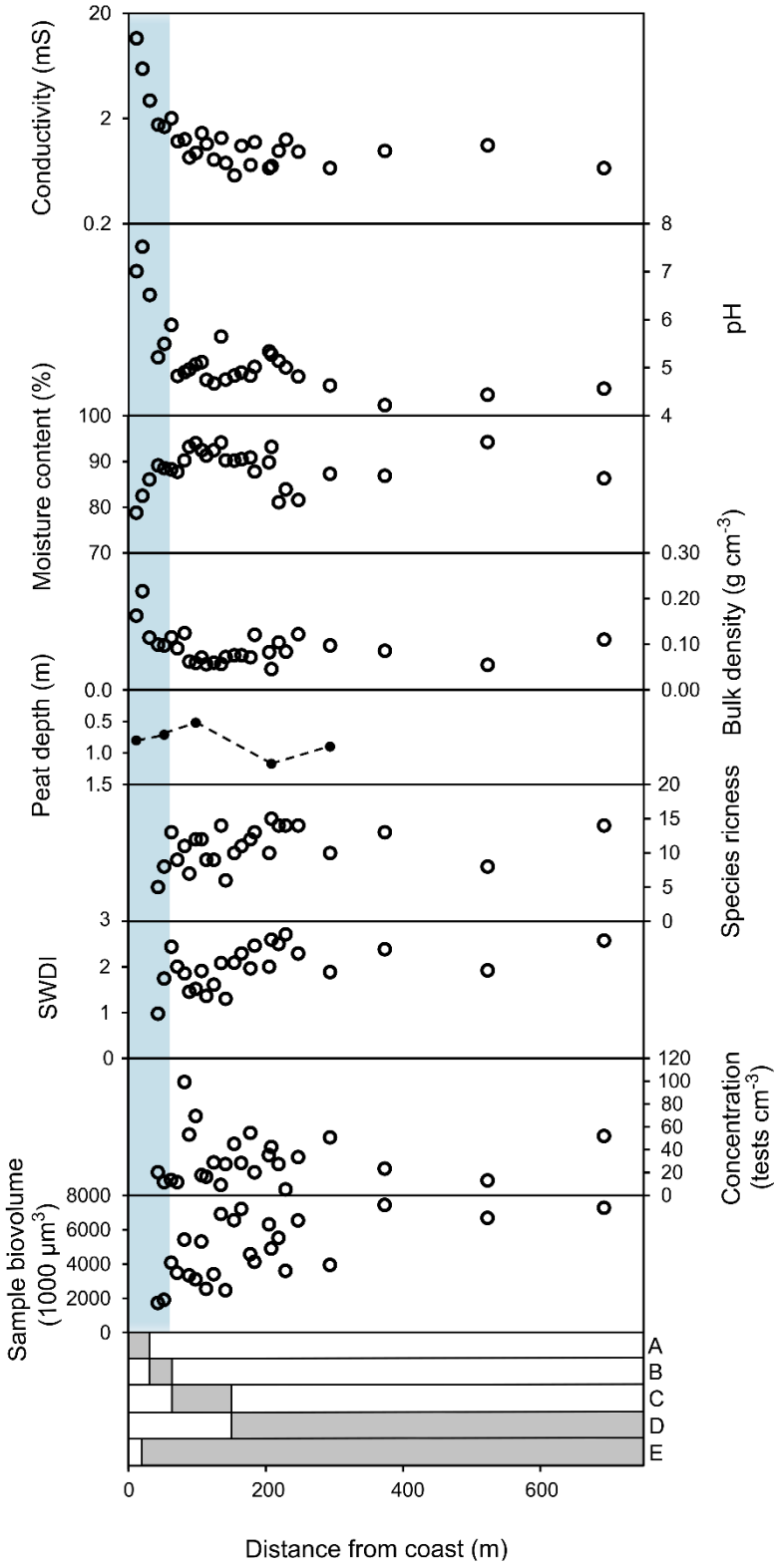

Figure 2 | Environmental and ecological variables recorded along the Kampkoppie surface transect, plotted against distance from the coast. Grey bars represent the extent of vegetation zones, where shading represents presence. Zones A-D represent vascular plants, and E represents bryophytes. Species included in each zone are presented in Table.S2 (Supplementary Information). Blue shading represents the section of the transect within ~75m from the coast, where concentrations of oceanic base cation input are high. Species richness is measured in the number of species recorded per station. SWDI refers to the Shannon-Weiner diversity index. Sample biovolume refers to the estimated biovolume of tests in the sampled population (n=100) at each station.

Results:

Environment

To identify variability in the environment inhabited by testate amoebae, we measured pore-water conductivity and pH, litter moisture content and bulk density (Fig.2). One sample (29) was omitted because moisture obtainable from the monolith core was insufficient for testing. Conductivity, which we interpret as a proxy for salinity, varied from 0.58-11.67 mS cm^{-1}. At most stations conductivity was low, mean 1.70 ± 2.21 mS cm^{-1}. Measurements of pH reflect the same pattern of variability, mean 5.19 ± 0.75, and ranged from acidic to neutral. The highest pH (7.52) was recorded at station 2, and the lowest (4.22) at station 26. Predominantly acidic conditions (pH ~4) typical of peatlands occur between ~300 m inland to the distal end of the transect.

Moisture conditions have previously been reported as a crucial control of testate amoebae colonisation and community structure[1,6,7,55]. Although conventionally measured as water-table depth, such data was unavailable for this study, and hence moisture content from the monolith cores was used in substitute. We propose that this provides a suitable indication of relative 'peatland surface wetness' between sites, since a high water table would raise soil moisture content, and both variables have been previously observed as co-dominant controls on testate amoebae distribution[56]. Variation in moisture between stations was minimal, assumingly due to the lack of microtopographic heterogeneity on the peatland surface and the flat plateau that it covers. The average was high 88.69 ± 4.17% typical for an accumulating peatland. Moisture content is broadly constant along the transect, excepting the first 100 m from the coast where values decrease. Bulk density of surface organic litter exhibits little change along much of the transect (mean= 0.0926 g cm^{-3}) but increases at the coast where sand grains are present at the surface (Fig.2). Peat depth, measured from core sampling was shallow (0.52-1.17 m).

Zonation of vegetation reflects the succession of a halophilic coastal community to non-halophilic species and increasing vegetation diversity with distance inland. In total 12 vascular plant species were present. The first 50 m from the coast is dominated by a small number of species; *Crassula moschata, Cotutla plumose,* and *Sagina procumbens*. Further inland, diversity increases and the coastal

community is replaced with species including *Ranunculus biternatus, Agrostis megallanica, Juncus scheucherioides, Azorella sellago, Montia fontana*. At the distal end of the transect (~200 m) the species *Blechnum penna-marina, Acaena megallanica, Uncinia compacta,* and *Lycopodium magellanicum* are also present. Bryophytes are present throughout the entire transect, excepting stations 1-2, located in the narrow band approximately 20 m from the coast.

Testate amoebae species composition and biomass

Testate amoebae analysis was conducted on all available samples (n=28) with accompanying environmental data. However, tests were either absent or of insufficient concentration in three of the samples (stations 1-3) and hence these samples were omitted from further analysis leaving 25 samples for inclusion in analysis. These samples revealed a testate amoebae fauna of 35 taxa, belonging to 18 genera (Table.1). Omitting types or unidentified taxa, which cannot be matched to the existing species list[36], twelve species are reported for the first time on Marion Island. We could not identify five taxa to the species level under the light microscope, and several taxa are the subject of taxonomic uncertainty. A new taxonomy, with illustrations of all species identified and accompanying identification guidance notes, was developed for this project [*Presented in Chapter 6*].

The genera *Argynnia* and *Euglypha* had the highest species number in the Marion Island population (Table.1), although the genus *Pseudodifflugia* dominated total relative abundance. Following this, the most dominant taxa were; *Pseudodifflugia* type 1(29.68%, found in 24 samples), *Pseudodifflugia* type 2 (7.16% in 19 samples), *Argynnia dentistoma* (6.64% in 20 samples) and *Euglypha tuberculata* (5.04% in 21 samples). The population is dominated by this small number of common species, with 16 of the 35 recorded species of relative abundance <1%.

The species richness of individual samples varied between 5 and 15, for stations 4 and 21 respectively (mean 11 ± 3 species per sample). Overall diversity was high (SWDI H: 2.00 ± 0.45), greatest at station 23 (H = 2.72), and lowest (H = 0.98) at station 4. SWDI values display a trend of broadly increasing values with distance inland from the coast, mirroring the trend in species richness.

Table 1 | Taxa recorded in the salt-influenced peatland at Kampkoppie (Marion Island) arranged by genus, estimated biovolume of an individual of each test, and relative abundance of each species in population, with total abundance of each genus in bold.

Genus	Species	Taxon Code	Biovolume (µm3)	Relative abundance species / **genus** (%)
Apodera	*vas*	Ap.va	110,000	**3.32**
Arcella	*arenaria*	Ar.ar	59,582	0.72 / **0.92**
	discoides	Ar.di	86,176	0.20
Argynnia	*caudata*	Ar.ca	80,960	2.12 / **9.52**
	dentistoma	Ar.de	86,827	6.64
	spicata	Ar.sp	195,000	0.12
	vitraea	Ar.vi	143,360	0.64
Assulina	*muscorum*	As.mu	21,432	3.12 / **3.12**
Centropyxis	*aerophila*	Ce.ae	76,700	1.04 / **4.84**
	constricta	Ce.co	113,900	0.92
	spp.1	Ce.spp.1	103,040	1.48
	spp.2	Ce.spp.2		1.40
Certesella	*certesi*	Ce.ce	211,500	4.52 / **5.32**
	certesi Type 1	Ce.ce.t1	234,360	0.36
	certesi Type 2	Ce.ce.t2		0.44
Corythion	*dubium*	Co.du	10,816	**3.44**
Cyclopyxis	*eurystoma*	Cy.eu	18,043	**0.88**
Difflugia	*globulosa*	Di.gl	18,043	3.68 / **5.64**
	pristis	Di.pr	75,309	0.08
	pulex	Di.pu	17,000	1.88
Euglypha	*laevis*	Eu.la	5,040	2.00 / **11.24**
	rotunda	Eu.ro	7,380	2.64
	strigosa	Eu.st	92,160	1.56
	tuberculata	Eu.tu	97,280	5.04
Heleopera	*petricola*	He.pe	132,000	**0.60**
Microchlamys	*patella*	Mi.pa	8,675	**4.88**
Pseudodifflugia	*spp.1*	Ps.spp.1	8,352	29.68 / **37.04**
	spp.2	Ps.spp.2	8,424	7.36
Pyxidicula	*spp.1*	Py.spp.1	36,317	1.60 / **1.72**
	operculata	Py.op	8,064	0.12
Quadrulella	*symmetrica*	Qu.sy	95,481	**4.08**
Tracheleuglypha	*dentata*	Tr.de	24,877	**1.04**
Trinema	*enchelys*	Tr.en	12,418	1.04 / **1.84**
	lineare	Tr.li	14,326	0.80
Valkonovia	*elegans*	Va.el	15,312	**0.56**

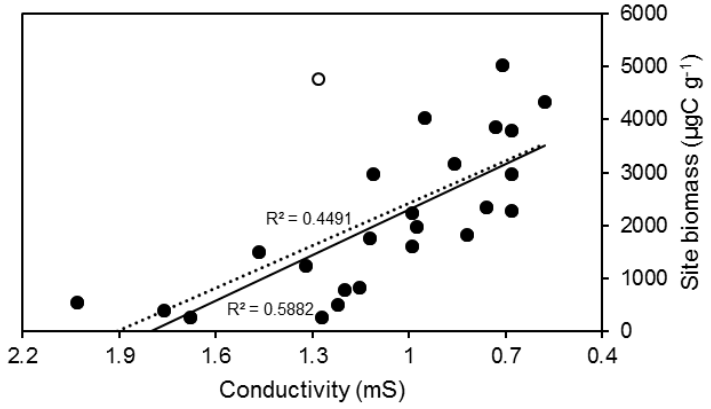

Figure 3 | Regression of estimated testate amoebae biomass and conductivity. Hollow marker indicates a significant outlier. Dotted trend line includes the outlier, and the solid line shows the improvement with the outlier removed from the dataset.

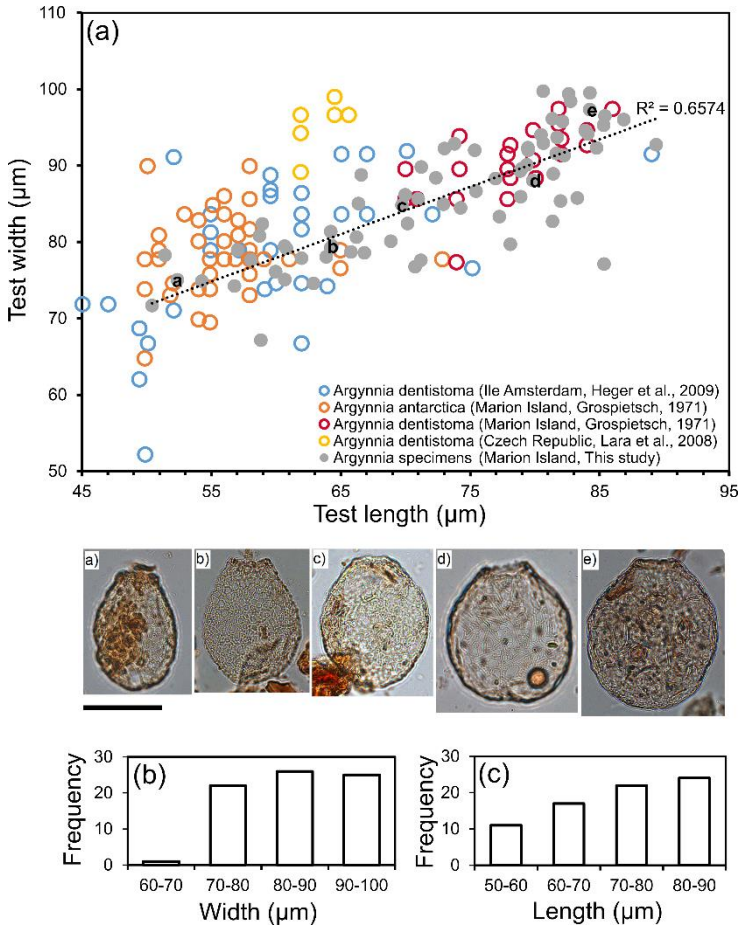

Figure 4 | Morphometry of the species *A. dentistoma.* a) Plot of width-length characteristics of individuals recorded in this study (grey) and the literature (other coulours), showing that individuals show gradational change. Letters refer to microphotographs of individuals (presented below). b and c) frequency plots of width and length classes, demonstrating that there is no evidence of clustering between different size fractions.

Mean concentration of tests per sample was 32,519 tests cm^{-3} or 413,828 tests dry g^{-1} and displayed no significant trend along the transect. We combined concentration values with measurements of test-volumes for individual species to estimate carbon biomass. Average biomass including all sampling stations was 2098 µg C dry g^{-1}. Regression analysis revealed a strong negative correlation between biomass and conductivity (Fig.3).

Morphometry of the *Argynnia* complex

The *Argynnia* complex in the sampled population was separated to isolate individuals of the species *A. dentistoma* to assess the hypothesis that *A. antarctica* is a distinct species. *A. spicata* and *A. caudata* were identified by the presence of spines on the test, and *A. vitraea* separated by size, test structure and aperture appearance. In total 74 well preserved individuals which matched the literature descriptions of *A. dentistoma* and *A. antarctica* were isolated for analysis. The mean length and width of the individuals was 85.79 ± 7.75 µm and 72.66 ± 10.14 µm respectively. Distribution of length and width was constant throughout the size fractions, without evidence of clustering (Fig.4).

Testate amoebae response to environmental gradients

CCA axis 1 (eigenvalue = 0.441) and 2 (eigenvalue = 0.273) explain 18.8% and 11.6% of the variance in the testate amoebae data respectively. Cumulative percentage variance of species-environment was 50.2% and 81.3% for axis 1 and 2 respectively. Conductivity is the environmental variable most strongly correlated to axis 1 (0.72), distance from the coast and moisture are most correlated with axis 2 (0.66 and 0.63 respectively) (Fig.5). The hypothesised link between testate amoebae distribution and inputs of salt spray can be accepted (i.e. changes in bog-surface conductivity can be predicted from testate amoebae assemblages).

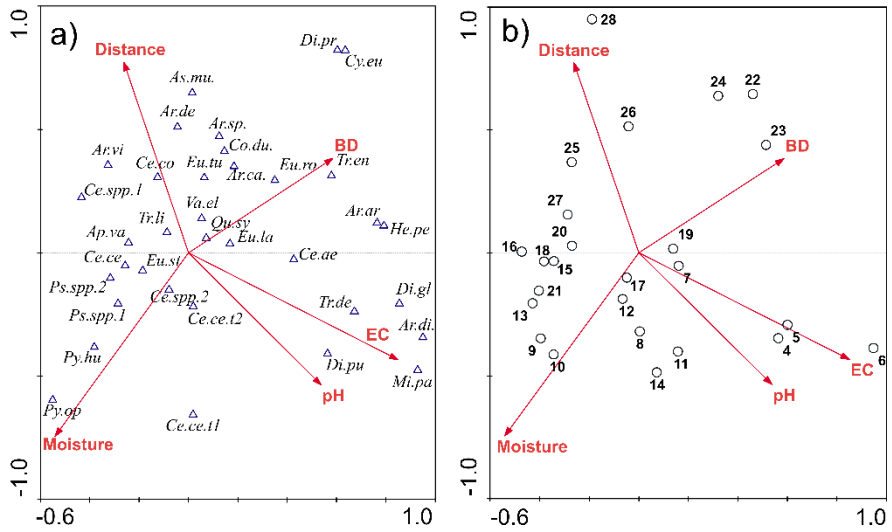

Figure 5 | CCA of testate amoebae from the transect with environmental variables (Distance from the coast, moisture content, pH, bulk density (BD) and conductivity (EC). a) species-environment, b) sample-environment. A key to the taxon codes is given in Table 1.

Statistical transfer function models are used to convert assemblage data into a prediction of bog-surface conductivity. Maximum likelihood (RMSEP = 0.3008, R^2 = 0.4523, skill = 31.52) was the best performing model in our exploratory analysis. Model performance was improved by screening the dataset, which removed 8 samples with residuals larger than 20% of the range in observed conductivity values (0.29 mS). This left a total of 16 samples for inclusion in the transfer function. After screening, model performance was high (RMSEP = 0.1666, R^2 = 0.7612, skill = 74.95), i.e. model predicted conductivity values generally compare well to the observed values of pore-water conductivity although the model is poorer at low salinity, a trend which is obscured when the data-set is screened (Fig.6). Plotting the residuals shows that there is no significant trend to indicate that the model would under or overestimate conductivity values towards either end of the sampled gradient.

Species optima and tolerance of testate amoebae to conductivity were calculated by weighted averaging (Fig.7). Although the sampled gradient of conductivity is small, it is apparent that different species of testate amoebae have preferences for different conductivity conditions. Species tolerances are large, and therefore indicate that most species have relatively wide ecological amplitudes.

Discussion:

Unfortunately, despite recent attention[37,57–61], the true diversity of testate amoebae that colonise peatlands of the sub-Antarctic remains only vaguely known. This study is the first to report the ecology of testate amoebae from a peatland on the west coast of Marion Island which is influenced by oceanic base cation input, and to examine the proposed application of these microorganisms as a proxy for documenting SHWW behaviour in the Late Quaternary.

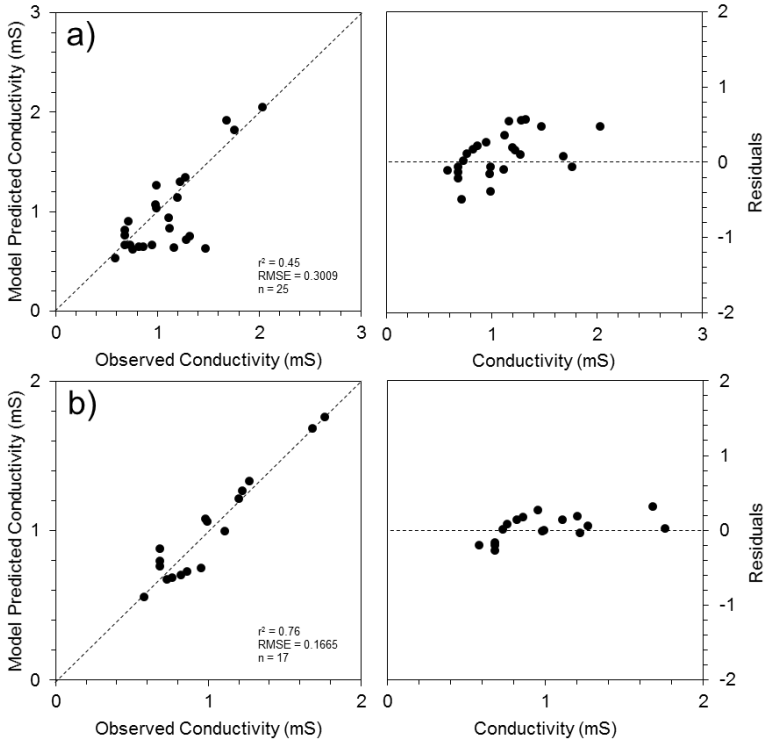

Figure 6 | Plots of observed versus estimated surface conductivity for the maximum likelihood transfer function model (left) **and residuals** (right). a) full dataset, b) screened dataset with outliers with residuals >20% of the range in observed conductivity values removed.

We attribute the observed gradient of conductivity and pH, across the peatland, to variable deposition of oceanic base-cations onto the surface. Mean pH and conductivity exceeds values expected in most peatlands because of continual oceanic ion enrichment by wind-generated spray, precipitation and fog, which together can deposit oceanic salts kilometres inland of the coast[62,63]. Both pH and conductivity measurements are highest at the coast and decrease with distance inland. This is because exposure to onshore winds and distance to the spray-source governs the input concentration of salts (i.e. concentrations increase with proximity to the coast and/or during periods of stronger wind). Analogous patterns of conductivity are also recorded on other sub-Antarctic

islands inside the westerly wind-belt[15,64].It has been suggested that because of this relationship, the gradient can be used as a modern basis for reconstructing past changes in westerly wind strength over the Southern Ocean[15,64]. It also remains possible that other factors affecting pH and conductivity, such as run-off and tephra deposition[42] are partly controlling the variability along the transect.

Testate amoebae diversity

Identification of species on Marion Island was hindered by the paucity of region specific literature, and so we developed a new taxonomy to aid standardisation with future research. Our taxonomy is based on features recognisable during routine counting under a light microscope. Therefore we acknowledge that the observed diversity is likely an underestimation, and has yet to be tested by genetics.

Species diversity is an important factor governing the successful application of testate amoebae in palaeoenvironmental studies. Populations with low diversity and dominant cosmopolitan generalist species, provide insufficient environmental information and has limited the application of testate amoebae based transfer functions for moisture on the Antarctic peninsula[44]. We identified 35 taxa (28 Species, 2 types and 5 unidentified at the species level). The population contains many small cosmopolitan species shared with other peatland regions around the world (e.g. *Trinema* spp*., Assulina muscorum,* and *Corythion dubium*). It is also dominated by a small number of common taxa; twenty-two taxa occurred with relative abundance of >1%, but only one taxa had an abundance >20%. The testate amoebae fauna is similar to other sub-Antarctic islands, with 89% of species previously identified, however unlike most existing studies we did not identify any species from the genus *Nebela.*

The only existing record of testate amoebae fauna on Marion Island used samples taken in close proximity to the research station on the east coast, and included mosses, soils and areas influenced by marine birds and animals[36]. Adding previously unidentified species from this study (12), and accounting for incorrectly identified taxa in the existing study[36], the best estimate of total diversity on Marion Island is increased to 64 known species. The term estimate is used because; (1) a number of species may have been identified differently between studies (e.g. *A. caudata* could have been confused with *N. playfairi* var. *elongata*),

and (2) some species may have been overlooked via misidentification or insufficient sampling of the environments which they inhabit. The latter is most likely to be a major limitation of our knowledge of testate amoebae diversity on the island.

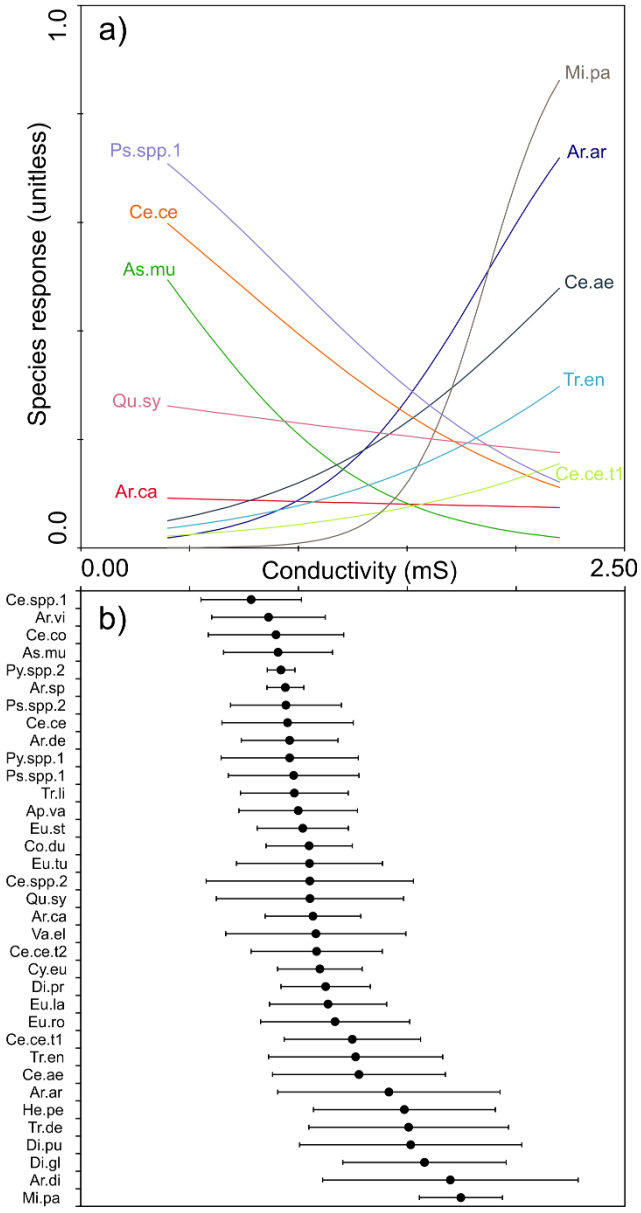

Figure 7 | Responses of individual testate amoebae species to conductivity conditions (a) species response curves for selected species, and (b) optima-tolerance plot for all identified taxa. Markers represent species optima, and error bars show the range of tolerated values. A key to the taxon codes is given in Table 1.

Since our samples were all from salt influenced soils, and previous sampling was also limited in terms of physiography and vegetation of areas included and

number of samples, we expect true diversity to increase if more in-depth studies are carried out in the future.

It has been proposed that diversity of testate amoebae (and diatoms[65]) decreases with latitude poleward (climatic severity), through the sub-, maritime-, and continental-Antarctic[35] . Our updated species list from Marion Island confirms the broad applicability of this trend for testate amoebae (i.e. recorded diversity is higher than the continental-Antarctic). However, at the scale of the sub-Antarctic, recent and more complete datasets from several islands suggest an increasingly complex pattern where climate severity, as well as biogeography are influential. Indeed, the simple linear trend explains variability throughout the sub-Antarctic latitudinal range poorly.

Testate amoebae diversity recorded in higher and colder latitudes of the sub-Antarctic, for example South Georgia (87 documented species[59]) exceeds Marion Island after addition of previously unreported species. There are a number of factors which can explain this. Firstly, South Georgia has a much larger land-area (4000 km^2 South Georgia, 300 km^2 Marion Island) and therefore potentially more diverse habitats to aid speciation, although it also has a significant extent of permanent ice-cover which limits areas suitable for colonisation. Secondly, whilst both islands have similar isolation from continental landmasses (~1,900 km), South Georgia is located downwind of South America, and therefore passive transport of testate amoebae by the aligned westerly winds and recolonization after decline is more likely there. The position of Marion Island south of its nearest continent (Africa), means that the zonal circulation of the westerlies effectively blocks dispersal of testate amoebae southward toward the island, and therefore isolation is greater. Larger species, too heavy for passive transport by winds can be carried over long distances by birds. Bird populations with different habitat preferences and geographical ranges inhabit both islands; this could provide a valid distributional pathway and further complicate diversity patterns. An important caveat of these conclusions is that current studies are often incomplete and lack taxonomic standardisation. This means that the testate amoebae diversity of each island is not yet known with certainty, and future research will benefit from increasing the detail, reliability and number of diversity reports.

Comparing the species list from this study against the limited number of modern studies from the sub-Antarctic[36,37,57–60], maritime Antarctic[61,66], and Antarctic

Peninsula[8,44] we report here three species not identified previously in the region. Two of these species are from the genus *Argynnia*; *A. spicata* and *A. vitraea*, and the other is *Quadrulella symmetrica*. A possible explanation is that *A. vitraea* may have been amalgamated into a *A. dentistoma* group in the past due to its morphological similarity, as it has in taxonomies for other regions of the world[7]. Likewise, *A. spicata* is likely to have been confused with *A. caudata* and previously categorised as *Nebela playfairi*, which has been reported.

The new recording of *Quadrulella symmetrica* in the Antarctic region is surprising because the species has a distinctive test that cannot be misidentified easily, and therefore should have been identified by previous studies if it was present. Also, suitable habitats to match known preferences are available on the sub-Antarctic islands (i.e. wet mosses, water, streams, organic litter and soil[67]). There are a number of possible explanations for occurrence of the *Q. symmetrica* in this study. One explanation is that the species might actually require moderately saline or high pH conditions. However, although it has been numerously reported in salt-marshes[10,68] , there are also records from other less saline habitats[2,3,7,50]. Additionally, our optima-tolerance model does not suggest a particular preference for strongly saline environments that might have been omitted from other studies. Laboratory error within our study can also be ruled out as we find a significant relative abundance (7th most abundant, 4.08%, 16 of 25 samples). Suggestions of a limited biogeographical distribution are also unfounded, the species size enables it to be dispersed widely, especially considering the appearance of much larger species (e.g. *Apodera vas*) in the population (Table.1).

Climatic change is a possible reason for the discovery of *Quadrulella symmetrica* in this study. Marion Island has experienced rapid temperature warming combined with significant precipitation decline in the decades since the first survey in 1971[36] (Fig.S3). It could be hypothesised that these changes represent an amelioration for the species, perhaps linked to food availability, allowing it to colonise the island for the first time, potentially after introduction as an invasive species. Future research would benefit from the collection of more samples to constrain the environmental preferences of the species on Marion Island and use of sediment cores to confirm whether the timing of first occurrence is recent, to more fully address this question.

Biogeography

Although existing literature is sparse, the testate amoebae assemblage identified in the samples is similar to freshwater species on Île de la Possession[60] 1,000 km to the East, with 17 of 28 (61%) species shared. Soil assemblages from the Île de la Possession are also similar 16 of 28 (57%) of species shared[57], and mosses on South Georgia, 5,000 km to the West, with 15 of 28 (54%) of species shared. Of the species identified from the Antarctic peninsula a large proportion of the species were also found in the Marion Island population; 58%[44] and 71%[8]. The large number of shared species is an indication that the population is composed of many generalists able to inhabit the more extreme climate conditions.

Sub-Antarctic islands are considered a distinct biogeographical region[15,65], and the potential for endemism is high[69]. Marion Island is therefore a unique location to contribute data to the debate of whether testate amoebae are microorganisms which display a limited or cosmopolitan distribution. A crucial aspect of biogeographical research is understanding whether a species is absent from a location due to geographical restriction, rather than lack of habitat[5] or simply misidentification. We do not find evidence for endemism in the recorded population.

Endemism of testate amoebae is of interest both biogeographically, and also in the development of transfer functions because they are reliant on establishing exact information on the environmental controls of populations that is applicable across large geographical areas. The species *Argynnia antarctica*, has been suggested as endemic to Marion island[36], despite a lack of evidence to support the claim. Recently, evidence from Île Amsterdam[37], another remote island, has suggested that the species is in fact indistinct from *A. dentistoma* and therefore not endemic to the island. This is a significant example of taxonomic uncertainty leading to assumptions of the limited geographical distribution of some testate amoebae species. Our analysis of length-width measurements from individuals on Marion Island showed no evidence of clustering (Fig. 4), and therefore we suggest that *A. antarctica* is not a distinct species. Rather our data supports the conclusion that the species *A. dentistoma* displays gradational variability in shell size. However, length-width ratios could plausibly be considered insufficient evidence for separation of species, and therefore it does remain a possibility that

A. antarctica is actually distinct, although our shell size data does not support this.

Response of testate amoebae to environmental variables

This study highlights that there is potential for testate amoebae to be used to semi-quantitatively reconstruct past changes in bog-surface conductivity in sub-Antarctic peatlands, and therefore provide inferential evidence of past changes in SHWW behaviour. CCA indicates that conductivity is a key community structuring variable, and that an apparent threshold level of conductivity for testate colonisation exists.

The salt-nourished peatland at Kampkoppie is a suitable habitat for testate amoebae colonisation. Unfavourable environments typically have SWDI values <0.5, generally low test concentration, and are dominated by a limited number of taxa. We expect that such conditions occur in the samples that contained an insufficient concentration of tests. Four stations on the transect occurred in the category SWDI= 0.1-1.5, indicative of a stressed environment. The remaining stations had SWDI values ranging from 1.5->2.5 indicative of transitional and healthy testate amoebae faunas, with high diversity and no overall dominant species.

Estimation of testate amoebae biomass is a relatively underused technique that brings together average test size and abundance, and together with diversity and concentration can be used as an indication of microbial activity. Average biomass recorded along the transect is high, indicating that the level of salt-enrichment does not significantly limit testate amoebae activity. The average is more than double the value reported on Signy Island in the Maritime Antarctic[44]. We attribute this to Marion Island's: (1) more suitable temperature conditions for testate amoebae, (2) greater number of large species in the population, (3) greater concentration of tests, and (4) higher diversity of testate amoebae permitted by more ecological niches.

Regression of biomass against conductivity revealed a strong correlation (R^2=0.59). We propose that this trend is caused by a general decrease in average test size as salinity values rise (i.e. smaller species dominate when salinity is high). Average biovolume in the sampled population (n=100 tests) supports this interpretation by indicating that average test size is greater inland where

conductivity is lower (Fig.2). Therefore, we interpret biomass as an indicator of the community scale response of testate amoebae. The strong correlation demonstrates the potential for biomass estimates to be used as a separate predictor of bog-surface salinity.

At the individual species scale optima-tolerance, calculated by simple weighted averaging, show a distinct partitioning of testate amoebae species along the conductivity gradient. However, there is a lack of physiological data which can be used to explain our results. It may be hypothesised, that the high ionic gradient in saline environments make acquisition of nutrient and water to maintain osmotic pressure in the cytoplasm difficult, with cells becoming desiccated when exposed to such conditions. It is also possible that salinity conditions affect the trophic level below testate amoebae (i.e. bacteria, fungi, algae and other protozoa[45]). Test size partly explains diet preferences; small taxa consume bacteria and small microbes, larger taxa consume a larger range of food, although some large taxa may also be specialists for certain food types. The availability of these different trophic levels could explain the difference in size along the transect.

Transfer function caveats and directions for future research

The results of our exploratory transfer function development represent a proof of concept and are intended to inform the direction of future research. They emphasise the value of further validation and testing of this emergent area of testate amoebae research. However, they must also be interpreted cautiously within the caveats of our small number of samples and single study site. Indeed, the next step for further research is to develop a transfer function suitable for palaeoenvironmental reconstruction. This requires a training set with; (1) a far greater number of samples, (2) samples from other geographical locations (islands), and (3) more robust environmental data (e.g. *insitu* water table measurements). Further, a resultant transfer function would need to be proven with rigorous testing[70], and an understanding of test taphonomy in these environments must be developed.

Removal of samples with low testate amoebae concentration, reduced the range of conductivity values included in the training set for this study. This is a likely driver of the relatively weak predictive power of the transfer function model, since few samples of either high or very low conductivity are included (i.e. most

samples are clustered around the centre of the gradient (Fig.6)). The transfer function is therefore heavily reliant on three high-conductivity outlier samples (Fig.6). Analysis of a greater number of samples from similar conditions is needed to verify whether these communities truly represent higher conductivity conditions. Even the most distal sampling location from the coast is partially nourished with oceanic salt so there are no samples of very low conductivity included in the analysis. By simple extrapolation it could be predicted that diversity, concentration and biomass would continue to rise under such conditions, and more extensive future sampling will be needed to test this theory.

The threshold for testate amoebae colonisation quantifies the upper limit of conductivity values that they can be used to predict, and further testing is needed to establish whether this trend is distinct to Marion Island. Future research would benefit from collecting a greater number of samples across a greater range of conductivity values, to fill these data-gaps, although logistical and financial constraints on such fieldwork meant that this was not possible for this project.

Ongoing research is similarly seeking to use sub-Antarctic diatoms from lakes to provide a proxy for wind-speed though reconstruction of oceanic salt inputs[15,64]. Lake diatoms can be used to reconstruct a wider range of conductivity values compared to peatland testate amoebae because they tolerate saline conditions (4.5 mS[15], 1.5mS this study). However, it is apparent that the salinity record of peatlands and lakes differs. For the same mass of input salt and over short time-periods, lake conductivity will alter less than bog-surface salinity due to dilution with available freshwater. Therefore, if bog-surface salinity can be reconstructed accurately, either via the species or community level responses described in this study, peatlands could provide a more sensitive record of lower magnitude wind changes.

Future research will be needed to test the application on a fossil core. Hypothetical interpretation of changing assemblages through time would be based on the following logic sequence. A shift to more salt-tolerant species and biomass decline would indicate a period of strengthened westerlies (i.e. increased storminess) with increased inputs of oceanic salts and higher bog surface conductivity. Extreme strengthening of the westerlies could be interpreted from the disappearance of testate amoebae from the record. Less salt-tolerant species and higher biomass would indicate periods of weaker winds, when bog

surface salinity declines because of precipitation removing more ions by leaching than are input to the peatland.

Before such reconstructions can be carried-out an understanding of the preservation of testate amoebae in these archives will need to be established. Preferential preservation of different taxa is a problem for palaeoenvironmental studies as it can alter the species composition leading to erroneous interpretation[71]. A further avenue for research is the possibility of developing transfer functions for testate amoebae from lakes, to allow multi-proxy comparison with results from ongoing diatom based reconstructions[72]. This study also demonstrates that community level responses of testate amoebae (i.e. presence-absence and biomass) could also be useful proxies for bog-surface salinity if investigations of these trends at other sites prove that the same correlations exist elsewhere.

We argue that the observed response of testate amoebae to bog-surface salinity indicates substantial value of this new proxy to terrestrial SHWW reconstructions in the sub-Antarctic region, and further research must be prioritised.

Conclusion

We add to the known testate amoebae fauna of Marion Island, by investigating samples from a west coast oceanic base cation enriched peatland. The majority of the testate amoebae we identified had been previously identified in the sub-Antarctic, although we report three species for the first time. We do not find evidence of endemic species.

Like other sub-Antarctic islands, the coastal zone is enriched in oceanic salts due to spray driven by the southern hemisphere westerly winds, which decrease exponentially with distance. Conductivity is a key testate amoebae community structuring variable in this environment, and a transfer function to predict bog-surface conductivity was developed. In the future it is possible that this relationship could be used as part of a proxy to study past changes in westerly wind strength. This would involve application of the transfer function to testate amoebae communities in sediment cores.

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Supplementary Information

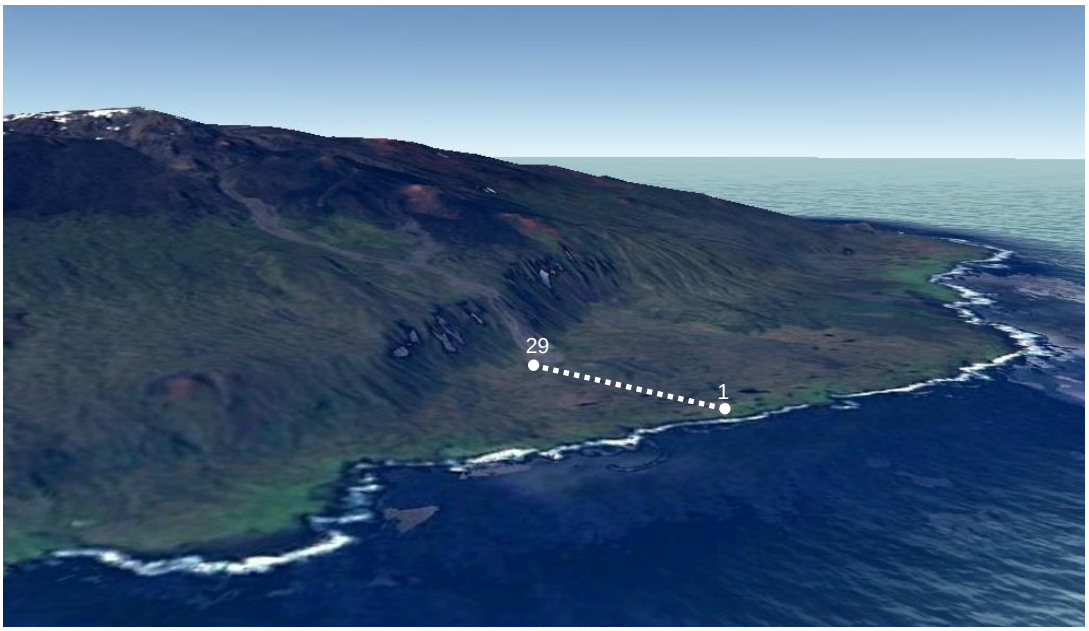

Figure S1 | Topography of the coastal terrace and geographical context of the peatland surface transect on Marion Island. Numbers refer to sample stations shown in Figure 1, and referred to in-text. Stations 2-28 are spaced at intervals along the transect. Source: Google Earth, 2016.

Table S1 | Biovolume estimates per species, with measurements used and test geometry class of each species. Dimensions given in italics are taken from literature sources and not direct measurements from the Marion Island population.

Species	Test geometry class	R	D	W	L	H	Source	Biovolume (μm^3)	Biomass (µg C)
Apodera vas	Ovoid			55	150	*20*	*Zapata and Fernandez (2008)*	110000	0.0121
Argynnia caudata	Ovoid			69	88	*20*	*Based on A. dentistoma*	80960	0.0089
Argynnia dentistoma	Ovoid			74	88	*20*	*NIES (2016)*	86827	0.0096
Argynnia spicata	Ovoid			113	130	*20*	*Based on A. dentistoma*	195000	0.0215
Argynnia vitraea	Ovoid			112	96	*20*	*Ogden and Hedley (1980)*	143360	0.0158
Assulina muscorum	Ovoid			38	47	*18*	*Ogden and Hedley (1980)*	21432	0.0024
Centropyxis constricta	Ovoid			67	85	*30*	*Based on C. cassis. NIES (2016)*	113900	0.0125
Centropyxis aerophila	Ovoid			59	65	*30*	*Based on C. cassis. NIES (2016)*	76700	0.0084
Centropyxis spp. 1 / 2	Ovoid			60	92	*28*	*Based on C. platystoma. NIES (2016)*	103040	0.0113
Certesella certesi	Ovoid			75	141	*30*	*Based on A. vas*	211500	0.0233
Certesella certesi Type 1 / 2	Ovoid			84	140	*30*	*Based on A. vas*	234360	0.0258
Corythion dubium	Ovoid			26	39	*16*	*Cash and Hopkinson (1909)*	10816	0.0012
Heleopera petricola	Ovoid			55	80	*45*	*NIES (2016)*	132000	0.0145
Pseudodifflugia Type 1	Ovoid			24	29	18		8352	0.0009

Table S1 continued.

Pseudodifflugia Type 2	Ovoid		26	27	18		8424	0.0009
Valkonovia elegans	Ovoid		29	44	*18*	*Based on A. muscorum*	15312	0.0017
Arcella arenaria	Saucer	42.5			*21*	*Microworld (2016)*	59582	0.0066
Arcella discoides	Saucer	45.5			*26.5*	*NIES (2016)*	86176	0.0095
Pyxidicula spp.1	Saucer	34			20		36317	0.0040
Pyxidicula operculata	Saucer	18.5			15		8064	0.0009
Microchlamys patella	Saucer	23.5			10		8675	0.0010
Cyclopyxis eurystoma	Hemispheric	20.5					261325	0.0287
Difflugia globulosa	Hemispheric	20.5					261325	0.0287
Difflugia pristis	Cyl-Ovoid		41	56			75309	0.0083
Difflugia pulex	Cyl-Ovoid		25	34			17000	0.0019
Euglypha laevis	Cyl-Ovoid		15	28			5040	0.0006
Euglypha rotunda	Cyl-Ovoid		15	41			7380	0.0008
Euglypha strigosa	Cyl-Ovoid		40	72			92160	0.0101
Euglypha tuberculata	Cyl-Ovoid		40	76			97280	0.0107
Quadrulella symmetrica	Cyl-Ovoid		41	71			95481	0.0105
Tracheleuglypha dentata	Cyl-Ovoid		26	46			24877	0.0027
Trinema enchelys	Cyl-Ovoid		19	43			12418	0.0014
Trinema lineare	Cyl-Ovoid		22	37			14326	0.0016

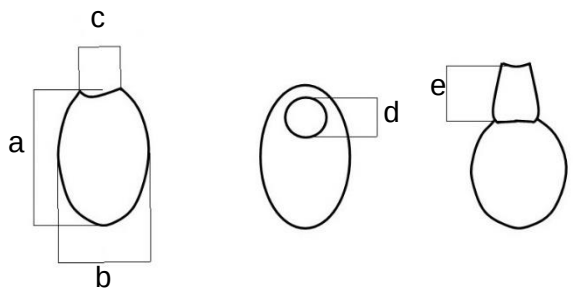

Figure S2 | Measured dimensions of different types of test recorded in this study. a) length, b) width, c) aperture width, measured for all species. d) Aperture length, measured for species with a hooded aperture, and e) neck length, measured for the species *Apodera vas* only. Measurements are presented in our new taxonomy.

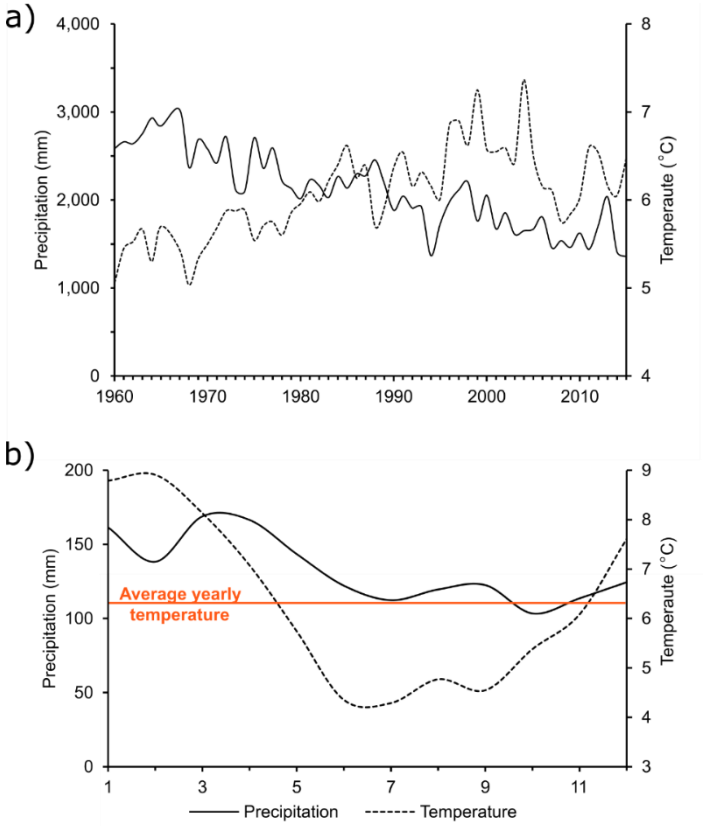

Figure S3 | Marion Island climate data from the South African National Antarctic Programme weather station on the east coast (NCDC, 2016). a) 1960-2015 average values of yearly precipitation and temperature, b) seasonal climate variability calculated from monthly means for the period 2010-2015. Numbers indicate month of the year (January 1 – December 12).

Table S2 | Vascular plant species included in each designated zone along the land-sea transect. Zonation is shown in Fig.2.

Zone 1	*Cotutla plumosa, Crassula moschata, Juncus scheucherioides, Montia fontana, Ranunculus biternatus, Sagina procumbens*
Zone 2	*Agrostis megallanica, Azorella sellago, Juncus scheucherioides, Montia fontana, Ranunculus biternatus*
Zone 3	*Acaena megallanica, Agrostis megallanica, Azorella sellago, Blechnum penna-marina, Juncus scheucherioides, Lycopodium magellanicum, Montia fontana, Uncinia compacta*

Supplementary References

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Chapter 6

Description notes and identification of peatland dwelling testate amoebae found at Kampkoppie (Marion Island)

Taxonomy of testate amoebae which inhabit; (1) areas within the land-sea continuum, (2) the southern hemisphere, and (3) peatlands which are not dominated by *Sphagnum* mosses, is relatively poorly documented compared to ombrotrophic peatlands. This is indicative of the frequent use of testate amoebae in ombrotrophic peatlands as indicators of hydrological conditions, and their less frequent use from other archives. Consequently there is a paucity of modern identification literature that is suitable for the amoebae present in peatlands on Marion Island and other islands in the sub-Antarctic. This has led to various examples of taxonomic confusion with examples of; species being identified interchangeably between studies, the force fitting of Northern hemisphere species names, and cases of misreported endemism. Further, this is compounded by the lack of any microphotographs presented by recent studies in the sub-Antarctic (e.g. Vincke et al., 2004; Vincke et al., 2006), meaning identifications cannot easily be independently verified. Development of a focussed taxonomic database is therefore important as a precursor to the identification of fossil specimens and the future usage of sub-Antarctic testate amoebae for palaeoenvironmental reconstruction.

The taxonomy that we developed is based on literature from peatlands, since this is the primary landscape classification of the study site. To maximise the amount of ecological information available, taxa have been separated to the lowest reasonable division possible for routine counting using a light microscope (Gehrels et al., 2001), and is based principally on simple shell characteristics and size. Where we believe that such groups do not represent either a true or currently identified taxa, we refer to them as ‘types’. The taxonomy should be viewed as a first step toward encapsulating the diversity of peatland testate amoebae in the sub-Antarctic, which will be developed as samples from more geographic regions and environments can be included.

***** denote species that have been identified for the first time on Marion Island.

Taxon Code ***Name*** Authority Figure Reference

Ap.Va. ***Apodera vas*** Certes 1889 (Figure. 1a-c)

Identification notes:

Highly distinctive, laterally compressed and lageniform test formed of two linked segments. One segment forms a funnelled neck and the other a rounded main body. The segments are delineated by a single constriction in the test that is approximately equivalent in length to half of the overall test width.

Colour ranges through dark brown, yellowish to hyaline. Most individuals observed in the Kampkoppie population were yellowish-hyaline. The aperture is bordered by a narrow smooth rim. Neck widens from the aperture, and lengths vary considerably, however they never exceed the length of the main body segment. Orientation of the neck can be slightly oblique relative to the main body, but this was not observed in the Kampkoppie population.

Observed individuals exhibited significant variability in neck length, suggesting that more than one 'type' may be present in the population, and possibly that this feature changes in response to environmental stresses. We did not divide the population by neck length in this study.

Evidence suggests that the species is limited to the Southern Hemisphere and tropics, and hence it is used to refute the theory of testate amoebae cosmopolitanism.

Relative abundance: 3.32%

Dimensions:

Length:	150 µm (132-169)
Width:	79 µm (62-101)
Aperture Width:	26 µm (22-30)
Neck length:	52 µm (26-62)
Width/Length:	1:1.9

Literature Dimensions:

Length:	111-234 µm	(Zapata & Fernandez 2008)
Width:	62-136 µm	
Depth:	25-86 µm	
Neck length:	37-98 µm	
Width/Length:	1:1.8	

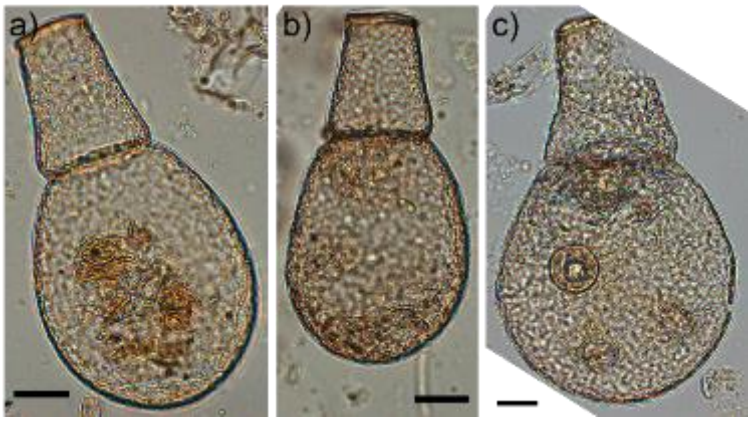

Figure 1 | ***Apodera vas*** (a-c) shown in broad lateral view. Scale bar is 20 µm.

Ar.ar. ***Arcella arenaria**** Greeff 1866 (Figure 2a-c)

Identification notes:

Generally dark brown, orange or yellow in colour. Test has a smooth surface without plates or imperfections. Aperture is moderately invaginated, central and surrounded by a smooth shallow lip. Small pores also surround the aperture, which range in abundance between authors (8-28), and therefore were not used as an identifying feature (Charman et al., 2000). Pores are often seemingly blocked (Ogden and Hedley, 1980).

Outline of the test is smooth, but not perfectly circular with slightly crumpled edges. Posterior surface of the test has a patterning of slight folds (Figure 2c), and a slightly domed shape.

Following Charman et al. (2000) *A. arenaria* is grouped together with *A. catinus* because they are deemed inseparable under light-microscope counting for palaeoenvironmental studies. However, other authorities indicate that aperture width could be a distinguishing feature (Ogden and Hedley, 1980; Microworld, 2016). Following this we have made the primary classification as *A. arenaria* for this study.

Relative abundance: 0.72%

Dimensions:

Length:	85 µm (64-106)
Aperture Width:	21 µm (14-29)
Diameter of aperture/Width	0.27 µm (0.16-0.55)

Literature Dimensions:

Length:	73-114 µm **C,O** 75-130 µm **M**	O = (Ogden & Hedley 1980) C = (Charman et al. 2000) M = (Microworld 2016)
Aperture Width:	22-33 µm **C** 23-37 µm **O** 12-28 µm **M**	

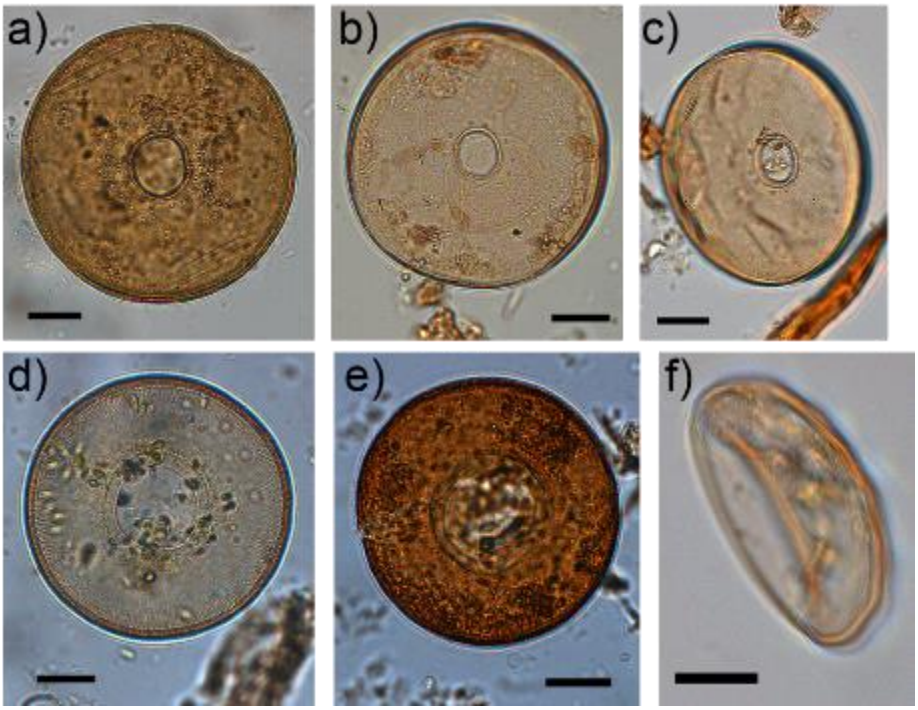

Figure 2 | a-c) *Arcella arenaria*: broad lateral view of two well preserved individuals showing the small pores which surround the central aperture (a-b) and cross-section (side) view showing the crumpled posterior surface of the test, and the smooth apertural rim, **d-f) *Arcella discoides***: broad lateral view showing wider aperture diameter compared to *Ar.ar.* Apertural plateau refers to the circular flattened section of test wall that surrounds the aperture (d-e) and cross-section showing the invaginated position of the aperture inside the centre of the test and its general saucer-like shape. Scale bar 20 µm.

Ar.di. ***Arcella discoides**** Ehrenberg 1872 (Figure 2d-f)

Identification notes:

Observed tests from Kampkoppie are often a deep-orange colour, ranging to light pink. Due to the more opaque, less glass-like appearance, the test is seemingly thicker than *A. arenaria*. Surface is also more irregular, and appears slightly pitted. Aperture is invaginated, central, but does not possess a thickened smooth lip around its circumference. Aperture width compared to overall test width is greater than *A. arenaria.* The aperture is approximately 1/3 of the overall test width.

Tests possess a slight plateau around the aperture which is generally visible, and the outline is less crumpled than *A. arenaria* in well preserved individuals. In cross-section the test is a flattened disc shape (Figure. 2f).

Ogden and Hedley (1980) comment that tests are delicate, and especially vulnerable to tearing. This is corroborated by this study, which rarely found a pristine individual.

Relative abundance: 0.20%

Dimensions:

Length:	91 µm (83-95)
Aperture Width:	31 µm (26-36)
Diameter of aperture/Width	0.35 (0.28-0.38)

Literature Dimensions

Length:	83-104 **O** 70-157 **M**	O = (Ogden & Hedley 1980) M = (Microworld 2016)
Aperture Width:	21-31 **O** 21-52 **M**	
Diameter of aperture/Width	0.30-0.45 **M**	

Ar.de. ***Argynnia dentistoma*** Penard 1890 (Figure. 3a-b)

Identification notes:

Most identification guides indicate that tests should be colourless, but many individuals from Kampkoppie were slightly yellow or brown. The test is made up of rounded plates arranged in an irregular but close fitting pattern over the entire surface. Sometimes plates are not visible and the test surface appears relatively smooth. Fragments of other particles such as diatom frustules are sometimes present.

The test tapers slightly toward the aperture which is composed of neatly arranged oval-shaped plates. The plates around the aperture help to differentiate this taxa from *N. parvula* which is similar in shape, but has a smooth aperture opening. The aperture is always aligned at right angles to the long-axis of the test. The shell plates do not overlap (Ogden and Hedley, 1980), instead they are held together with a porous cement, although this is not visible under a light-microscope. Size of the overall test and aperture width is a distinguishing factor between *Ar.de* and *Ar.vi.*

There is debate in the literature whether morphologically similar individuals from Marion Island reported by Grospietsch (1971) represent a separate species referred to as *Argynnia antarctica.* Our morphometric assessment does not support the view that the species are distinct.

Relative abundance: 6.64%

Dimensions:

Length:	88 µm (62-119)
Width:	74 µm (51-97)
Aperture Width:	25 µm (16-34)
Width/Length:	1:1.19

Literature Dimensions:

Length:	81-96 µm **O** 96 µm **L** 79 µm **H**	O = (Ogden & Hedley 1980) L = (Lara et al. 2008) H= (Heger et al. 2009)
Width:	58-80 µm **O** 64 µm **L** 60 µm **H**	

Ar.vi ***Argynnia vitraea**** Penard 1899 (Figure. 3c-d)

Identification notes:

Very similar in appearance to *Ar.de* but slightly larger in size, despite maintaining equivalent proportions. Shell is generally colourless, glassy and composed of oval to circular plates. Ogden and Hedley (1980), proposed that arrangement of shell plates could be used as a distinguishing feature of *Ar.vi*. Although some individuals from Kampkoppie do display slightly overlapping plates we found this feature inappropriate for species separation, especially for palaeoenvironmental purposes when tests may be poorly preserved.

We based our separation on test length and width, and that the outline of *Ar.vi* is often irregular with various bumps and a crumpled appearance. Compared with *Ar.de* the aperture is often messier, and not always aligned at right angles to the long-axis of the test.

Relative abundance: 0.64%

Dimensions:

Length:	112 µm (83-174)
Width:	96 µm (82-133)
Aperture Width:	32 µm (22-44)
Width/Length:	1:1.17

Literature Dimensions:

Length:	117-160 µm **O** 120-151 µm **C**	O = (Ogden & Hedley 1980) C = (Charman et al. 2000)
Width:	102-145 µm **O** 104-138 µm **C**	
Depth:	55-95 µm **O**	
Aperture width:	28-37 µm **O**	

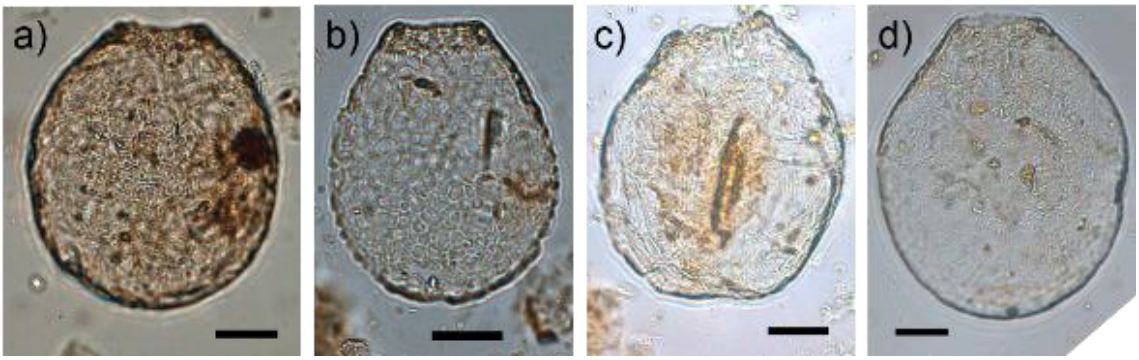

Figure 3 | *Argynnia dentistoma* (a-b), ***Argynnia vitrea*** (c-d). Scale bar is 20 µm.

Ar.ca ***Argynnia caudata**** Leidy 1879 (Figure 4a-c)

Identification notes:

Shell is ovoid in shape, with 3-5 horns located around the shell perimeter. Test is compressed, and often has visible plates around the aperture (Fig. 4a). Test composition appears to be similar to *Ar.de.* Diatom fragments are often distinguishable in the test structure.

We suggest that there has been possible confusion with the species *Nebela playfairi,* in the literature, based on microphotographs presented in (Grospietsch 1971).

Relative abundance: 2.12%

Dimensions:

Length:	88 µm (72-107)
Width:	69 µm (58-86)
Aperture Width:	24 µm (18-32)
Spine length:	15 µm (7-22)
Width/Length:	1:1.28

Literature Dimensions:

Length:	60-65 µm **O** 76-90 µm **M**	O = (Ogden & Hedley 1980) M = (Microworld 2016)
Width:	58-70 µm **M**	

Ar.sp. ***Argynnia spicata**** Wailes 1913 (Figure 4d)

Identification notes:

Similar in morphology to *Argynnia caudata,* but only found in the Southern Hemisphere (Swindles et al., 2014). It is differentiated because the shell wall is regularly shaped and doesn't taper toward points where the spines join. Also the test does not taper toward the aperture. The shell is comprised of near circular plates that interlock closely. The number of spines is greater than 5, as observed in the Kamkoppie population. Normally 7 spines are present, and they are always clustered in the lower half of the test. This taxa was very rare in the Kampkoppie population, and significantly smaller than the literature suggestion. Generally assumed to be larger than *Argynnia caudata,* but individuals sampled in this study were significantly smaller (Figure 4).

Relative abundance: 0.12%

Literature Dimensions:

Length:	120-140 µm	(Microworld 2016)
Width:	100-125 µm	

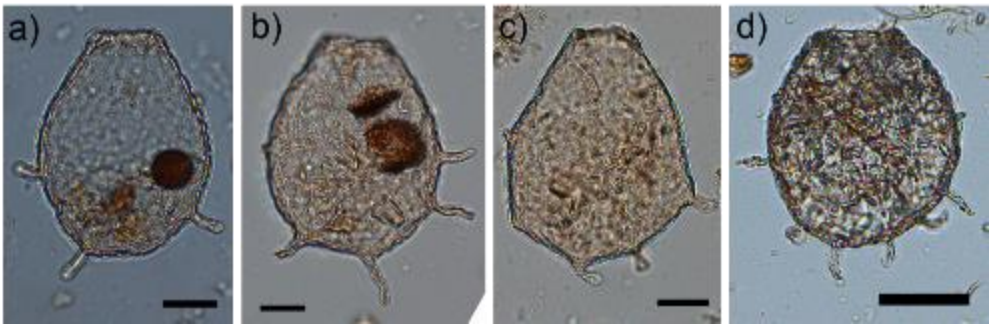

Figure 4 | ***Argynnia caudata*** (a-c), and ***Argynnia spicata*** (d). Scale bar is 20 µm.

As.mu. ***Assulina muscorum*** Greef 1888 (Figure. 5)

Identification notes:

Most striking identifying characteristic is the 'russet-brown' to pink-red colour of the test, although it can occasionally be colourless. Shell is comprised of oval plates which can appear slightly angular under a light microscope. The plates are arranged regularly and neatly in rows and all are equally sized. Sometimes the plates are very small. Plates overlap the aperture to give a toothed appearance. Colour often grades to transparent at the aperture. Test is smaller than *A. seminulum* which was not found in the Kampkoppie population.

Relative abundance: 3.12%

Dimensions:

Length:	47 µm (31-70)
Width:	38 µm (24-49)
Aperture Width:	14 µm (9-21)
Width/Length:	1:1.25

Literature Dimensions:

Length:	45-53 µm **O** 46-58 µm **C** 35-60 µm **G**	O = (Ogden & Hedley 1980) C = (Charman et al. 2000) G = (Gehrels et al. 2006)
Width:	32-48 µm **O** 34-44 µm **C** 25-40 µm **G**	
Depth:	18-22 µm **O**	
Aperture width:	12-18 µm **O** 16-20 µm **C**	

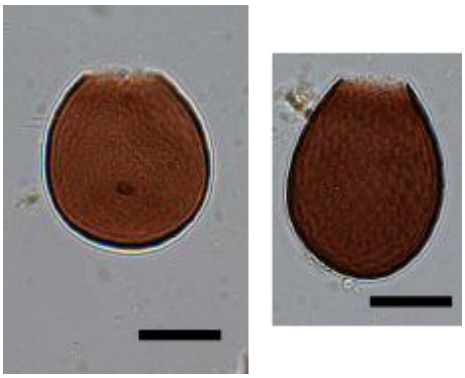

Figure 5 | ***Assulina muscorum.*** Scale bar is 20 µm.

Centropyxis complex

Taxa of the genus *Centropyxis* within the Kampkoppie population could not be identified confidently, and appear to display gradational changes of morphology. Biometric analysis, based on test length and width, indicate two main groups (Figure 6), although microphotographs imply a greater degree of variability (Figure 7). Further observation will be needed to accurately standardise the identification of species in this genus.

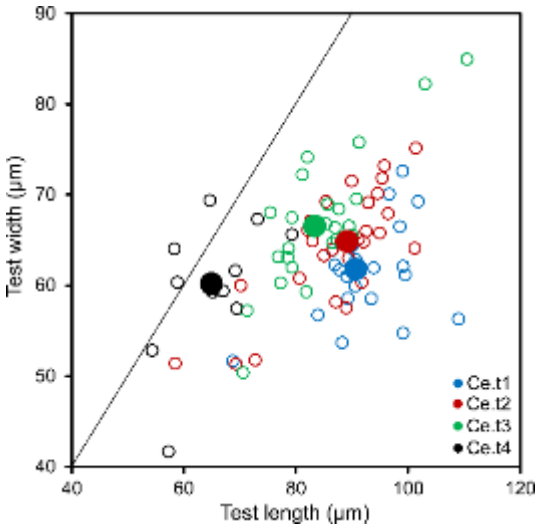

Figure 6 | Biometric data of *Centropyxis* specimens from Kampkoppie. Solid markers indicate ideal individuals of each taxa constructed from the median of values shown as hollow markers. Line indicates length : width ratio of 1:1 (i.e. circular test shape). Key; Ce.t1 = *Centropyxis constricta,* Ce.t2 = *Centropyxis* spp.1 Ce.t3 = *Centropyxis* spp. 2, and Ce.t4 = *Centropyxis aerophila* .

Ce.co ***Centropyxis constricta*** Ehrenberg 1841 (Figure. 7a-c)

Identification notes:

Tests are differentiated in this study due to its pill-like shape, with an aperture that is generally circular in shape. Test does not increase in width along long-axis. Aperture is not kidney shaped like *Ce.spp.2*.

Relative abundance: 0.92%

Dimensions:

Length:	92 µm (52-109)
Width:	60 µm (26-73)
Aperture width (x):	36 µm (22-44)
Aperture width (y)	24 µm (20-32)
Width/Length:	1:1.52

Ce.spp.1 ***Centropyxis* spp. 1** (Figure. 7d-f)

Identification notes:

The test has a very similar shape to *Ce.co.,* although it is slightly rounder, and has a slightly more oval shaped aperture. We propose that these individuals are most likely an intermediate form between C*e.co.* and *Ce.spp.2.*

Relative abundance: 1.48%

Dimensions:

Length:	87 µm (58-101)
Width:	64 µm (51-75)
Aperture width (x):	36 µm (26-44)
Aperture width (y)	23 µm (15-30)
Width/Length:	1:1.37

Ce.spp.2 ***Centropyxis* spp. 2** (Figure. 7g-i)

Identification notes:

The test is separated from *Ce.co* based on its lower width:length ratio, and kidney shaped aperture.

Relative abundance: 1.40%

Dimensions:

Length:	85 µm (71-111)
Width:	67 µm (50-85)
Aperture width (x):	38 µm (23-51)
Aperture width (y)	21 µm (16-26)
Width/Length:	1:1.27

Ce.ae ***Centropyxis aerophila*** Deflandre 1929 (Figure. 7j-l)

Identification notes:

The test has a rounded and compressed shape, which differentiates it. Aperture is wide and oval.

Relative abundance: 1.04%

Dimensions:

Length:	65 µm (54-79)
Width:	59 µm (42-69)
Aperture width (x):	30 µm (21-36)
Aperture width (y)	16 µm (11-22)
Width/Length:	1:1.09

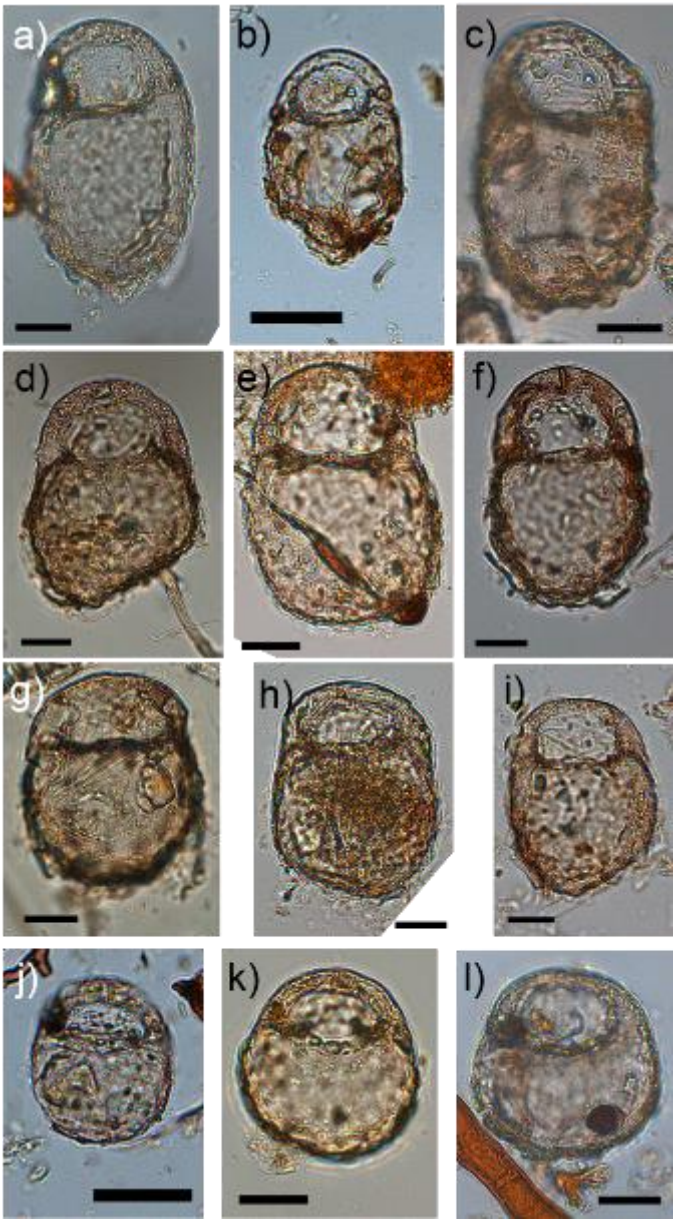

Figure 7 | ***Centropyxis constricta*** (a-c), ***Centropyxis*** **spp 1** (d-f), ***Centropyxis*** **spp 2** (g-i) and ***Centropyxis aerophila***. Scale bar is 20 µm.

Ce.ce. ***Certesella certesi*** Penard 1911 (Figure. 8a-c)

Identification notes:

The genus *Certesella* is restricted to the southern hemisphere, and suggested to be limited to areas that originally belonged to Gondwanaland.

The test is large and flask shaped with a distinct neck equivalent in width to the aperture, with parallel sides. The neck is covered by a network of small pores, and the apertural rim has a smooth secreted lip. Aperture rim is generally clear and distinctive under a light microscope. Aperture is slightly convex in shape relative to the test body. There is a single tooth-like structure inside the aperture, where the test thickens. Tooth can be used to differentiate it from *C. martiali*, which has two internal structures in the aperture neck.

Tests often have a slightly crumpled appearance, which makes it look delicate, whereas *C. martiali* looks more robust (e.g. images of both species published by Van Bellen et al. (2014)).

At the base of the neck two large pores are present at either side of the test. These pores are situated in depressions of the test surface, and are connected by an internal tubular structure. Pores range in size, but are always larger than those on the test neck, there are never more than 2. Test is colourless to light yellow-pink-brown.

Relative abundance: 3.88%

Dimensions:

Length:	141 µm (128-158)
Width:	75 µm (60-88)
Aperture Width:	29 µm (25-33)
Width/Length:	1:1.89

Literature Dimensions:

Length:	145-15 µm	(Microworld 2016)

Ce.ce.t1 ***Certesella certesi*** **Type 1** (Figure. 8d-f)

Identification notes:

The main body of the test is similar in morphology to *Ce.ce* but does not possess a distinct neck. Test is pyriform and tapers towards the aperture which has a thin, smooth lip. Characteristically, two large pores are present approximately half way along the test length, and are located very close the shell edge. Tests do not seem to possess a central tooth in neck toward aperture.

Numerous small pores are present in the test close to the aperture. Intermediate test shapes between the classifications were also recorded.

Relative abundance: 0.36%

Dimensions:

Length:	149 µm (144-151)
Width:	86 µm (76-96)
Aperture Width:	33 µm (27-36)
Width/Length:	1:1.73

Ce.ce.t2 ***Certesella certesi*** **Type 2** (Figure. 8g-i)

Identification notes:

The test appears to be a compressed version of *Ce.ce* with a shorter neck and more bulbous main body. Two large apertures are located where the neck joins the main body section, and small pores are found around the aperture.

It is possible that this type could be confused with *Nebela militaris* due to its similar test shape, however *Ce.ce.t2* is larger and has the characteristic pores (as described above). *N. militaris* can also be differentiated by its thick organic collar surrounding the aperture.

Relative abundance: 0.44%

Dimensions:

Length:	130 µm (123-139)
Width:	82 µm (74-89)
Aperture Width:	28 µm (23-33)
Width/Length:	1:1.61

Co.du. ***Corythion dubium*** Taranek 1871 (Figure. 9)

Identification notes:

The test is comprised of oval plates that were rarely visible in the Kampkoppie population. The aperture is sub-terminal and surrounded by a distinctive hood, and a serrated interior border. It is transparent-white and ephemeral in appearance. A relatively large difference in size between individuals was observed but test shape remains constant. Aperture shape ranges from oval to near circular.

Relative abundance: 3.44%

Dimensions:

Length:	39 µm (30-47)
Width:	26 µm (15-33)
Aperture width (x):	11 µm (7-14)
Aperture width (y):	7 µm (4-10)
Width/Length:	1:1.5

Literature Dimensions:

Length:	33-55 µm	(Ogden & Hedley 1980)
Width:	24-33 µm	
Aperture width:	9-17 µm	

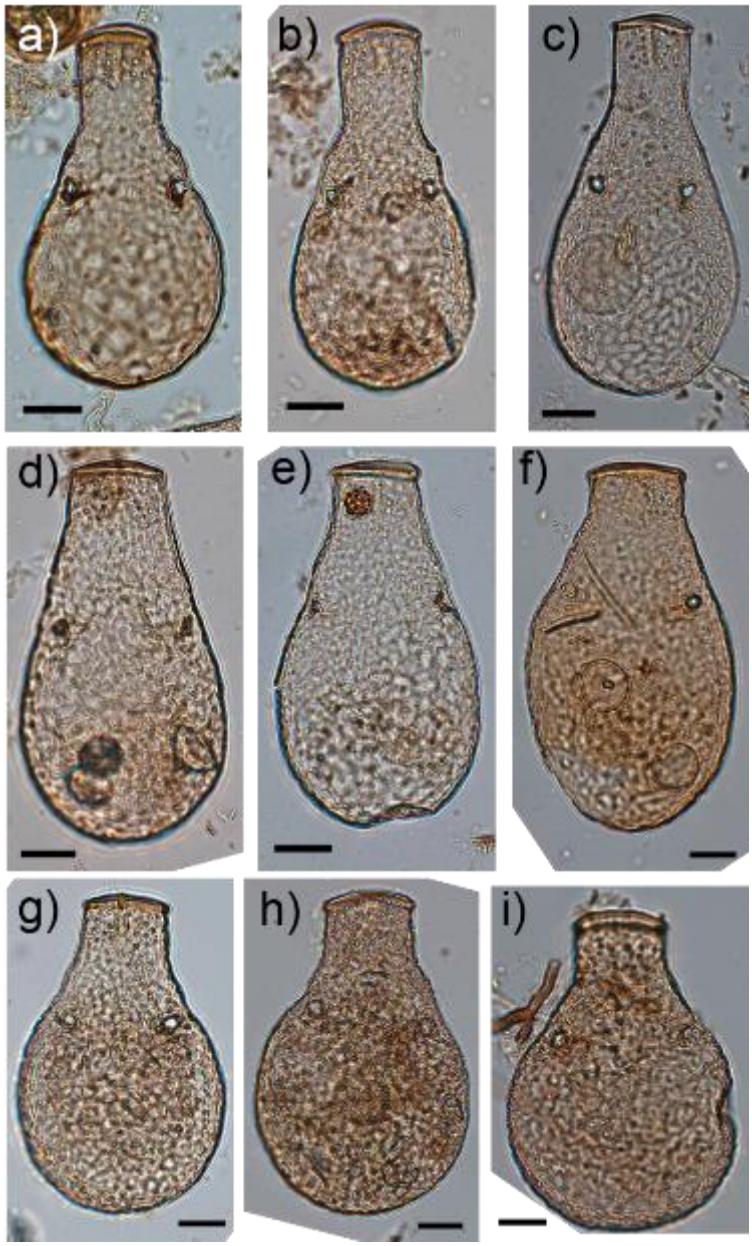

Figure 8 | ***Certesella certesi*** (a-c)**,** ***Certesella certesi*** **type 1** (d-f)**,** ***Certesella ceresi*** **type 2** (g-i)**.** Scale bar is 20 µm.

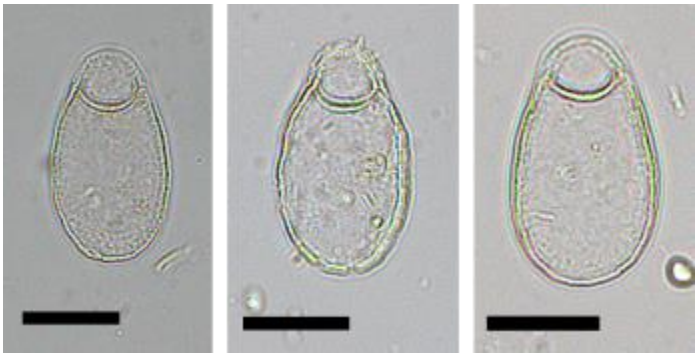

Figure 9 | ***Corythion dubium.*** Scale bar is 20 µm.

Di.gl. ***Difflugia globulosa**** Penard 1902 (Figure. 10e-h)

The identification remains uncertain and further observation will be needed to more accurately identify this taxa.

Identification notes:

Literature suggests that test dimensions should be larger than individuals measured from the population at Kampkoppie, but a smaller form has also been described. We differentiate from *Cyclopyxis eurystoma* because *Difflugia globulosa* is generally less hemispheric in cross-section, and the aperture is smaller relative to test width, however this separation is tentative since this could be a gradational characteristic.

Relative abundance: 3.68%

Dimensions:

Length:	40.54 µm
Width:	40.38 µm
Aperture width:	19.05 µm

Literature Dimensions:

Length:	91-119 µm	(Ogden & Hedley 1980)
Width:	79-113 µm	
Aperture width:	33-58 µm	

Cy.eu ***Cyclopyxis eurystoma**** Deflandre 1929 (Figure 10e-h)

The identification remains uncertain and further observation will be needed to more accurately identify this taxa.

Identification notes:

The test has a rough surface comprised of small particles, and is hemispheric in lateral view. Aperture is circular, terminal and surrounded by a smooth collar. Tests from the sampled population generally appeared brown-yellow in colour. In cross section the test is rounder than *Difflugia globulosa.*

Relative abundance: 0.88%

Dimensions:

Length:	41.25 µm
Width:	40.97 µm
Aperture width:	18.81 µm

Literature Dimensions:

Diameter:	69-80 µm	(Ogden & Hedley 1980)
Depth:	46-55 µm	
Aperture width:	34-46 µm	

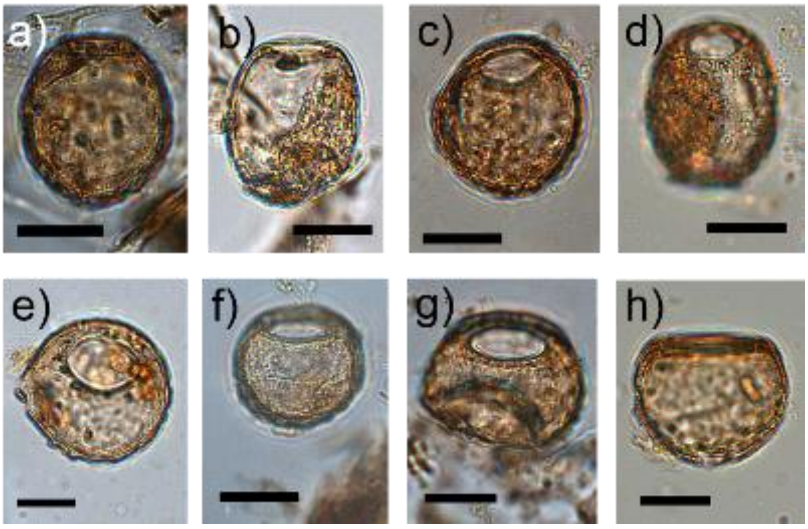

Figure 10 | ***Difflugia globulosa*** (a-d) and ***Cyclopyxis eurystoma*** (e-h). Scale bar is 20 µm.

Di.pr. ***Difflugia pristis**** Penard 1902 (Figure. 12a)

Identification notes:

The test is less pyriform and larger compared to *Difflugia pulex* (Figure. 11). Very few individuals were observed in the Kampkoppie population.

Relative abundance: 0.08%

Dimensions:

Length:	56 µm (51-61)
Width:	39 µm (36-43)
Aperture width:	20 µm (18-22)
Width/Length:	1:1.44

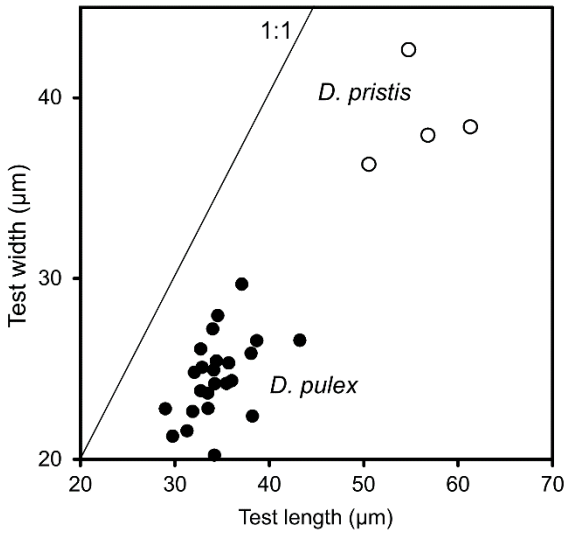

Figure 11 | Biometric data of *Difflugia* specimens from Kampkoppie, demonstrating the clear separation of the two species.

Di.pu. ***Difflugia pulex**** Penard 1902 (Figure. 12b-c)

Identification notes:

The test is pyriform, and narrows sharply at the aperture, which is narrow relative to the test width. It is composed of particles which gives it an untidy appearance.

Relative abundance: 1.88%

Dimensions:

Length:	34 µm (29-43)
Width:	25 µm (20-30)
Aperture width:	12 µm (10-15)
Width/Length:	1:1.4

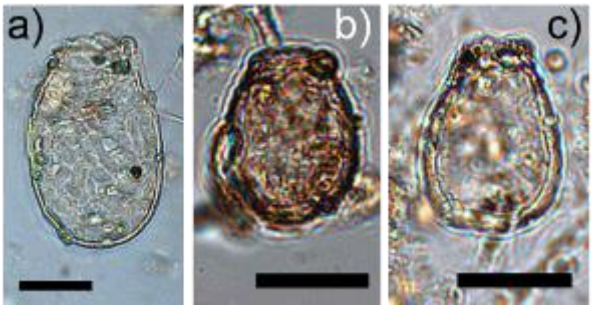

Figure 12 | ***Difflugia pristis*** (a) and ***Difflugia pulex*** (b-c). Scale bar is 20 µm.

Eu.la ***Euglypha laevis*** Ehrenberg 1845 (Figure. 13a)

Identification notes:

The test can easily be confused with *Euglypha rotunda* due to its similarity in appearance. Plates are rarely visible under a light microscope due to the small size of the test, and therefore it appears smooth. We based our distinction primarily on size and its smooth appearance.

Relative abundance: 2.00%

Dimensions:

Length:	28 µm (22-43)
Width:	15 µm (10-25)
Aperture width:	7 µm (5-16)
Width/Length:	1:1.86

Eu.ro ***Euglypha rotunda*** Ehrenberg 1845 (Figure.13c)

Identification notes:

The test is ovoid and with a similar structure to *Euglypha tuberculata*. It is composed of oval shaped plates which fit together closely. The aperture is

surrounded by toothed plates. It is differentiated from *Eu.tu.* due to its smaller size which is generally <50 µm.

Relative abundance: 2.64%

Dimensions:

Length:	41 µm (32-60)
Width:	27 µm (16-40)
Aperture width:	13 µm (7-21)
Width/Length:	1:1.55

Eu.st ***Euglypha strigosa*** Ehrenberg 1848 (Figure. 13b)

Identification notes:

The test is ovoid shaped and adorned with rhombus shaped plates. The plates fit together tightly in a neat pattern. It is differentiated from other *Euglypha* species by the presence of narrow siliceous spines which project from the test. Individuals from the Kampkoppie population were often poorly preserved.

Relative abundance: 1.56%

Dimensions:

Length:	72 µm (58-97)
Width:	44 µm (29-66)
Aperture width:	20 µm (14-34)
Width/Length:	1:1.64

Eu.tu ***Euglypha tuberculata**** Dujardin 1841 (Figure. 13d)

Identification notes:

The test is large and covered by tightly fitting scales which appear to be rhombus shaped. Toothed plates surround the aperture and give a jagged appearance. The shell is very similar to *Euglypha rotunda* but is separated in this study primarily by its larger size (>55 µm).

Relative abundance: 5.04%

Dimensions:

Length:	76 µm (55-95)
Width:	47 µm (26-70)
Aperture width:	21 µm (13-31)
Width/Length:	1:1.62

Literature Dimensions:

Length:	55-100 **M** 74-95 µm **O** 45-100 µm C	O = (Ogden & Hedley 1980) C = (Charman et al. 2000) M = (Microworld 2016)
Width:	36-51 µm **O**	
Aperture width:	18-21 µm **O**	

Figure 13 | ***Euglypha laevis*** (a), ***Euglypha strigosa*** (b), ***Euglypha rotunda*** (c) and ***Euglypha tuberculata*** (d). Scale bar is 20 µm.

He.pe. ***Heleopera petricola**** Leidy 1879 (Figure. 14 a-b)

Identification notes:

The test has a characteristic broad slit-shaped terminal aperture with a well-defined smooth rim. The width of the aperture is similar to the overall width of the test. The test is oval-shaped in lateral view and flattens slightly at the aperture.

Relative abundance: 0.60%

Dimensions:

Length:	80 µm (68-93)
Width:	55 µm (48-61)
Aperture width:	32 µm (27-39)
Width/Length:	1:1.47

Literature Dimensions:

Length:	76-84 µm	(Ogden & Hedley 1980)
Width:	51-57 µm	
Depth:	40-50 µm	
Aperture width:	31-34 µm	

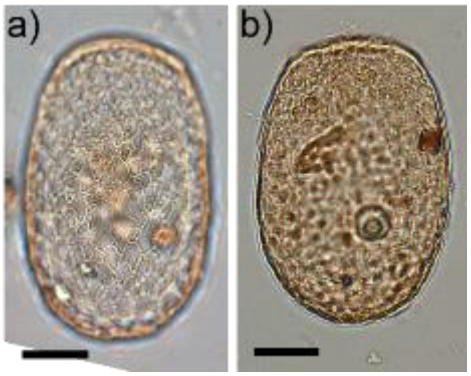

Figure 14 | ***Heleopera petricola*** (a-b). Scale bar is 20 µm.

Ps.spp.1 ***Pseudodifflugia spp.1**** (Figure. 15a-d)

Identification notes:

The test is smooth with some particles which often appear brown-grey in colour. Shell plates are not present. The test is very small, generally <30 µm in length. We observed very little morphological variability between individuals in the population. Test outline is well defined, and often crumpled slightly. It shares similarities with *Pseudodifflugia fascicularis*, although the prominent collar around the aperture is not present in this species. The aperture is narrow, terminal and surrounded by a short smooth collared neck. Highly abundant in the population at Kampkoppie.

Relative abundance: 29.68%

Dimensions:

Length:	29 µm (25-33)
Width:	24 µm (11-29)
Aperture width:	12 µm (9-25)
Width/Length:	1:1.2

Ps.spp.2 ***Pseudodifflugia spp.2**** (Figure. 15e-h)

Identification notes:

Very similar to *Ps.spp.1* but lacking collar around the aperture. Some individuals have small spines adorning the posterior end of the tests (Figure 15h).

Relative abundance: 7.16%

Dimensions:

Length:	27 µm (20-33)
Width:	26 µm (17-29)
Aperture width:	13 µm (9-17)
Width/Length:	1:1.07

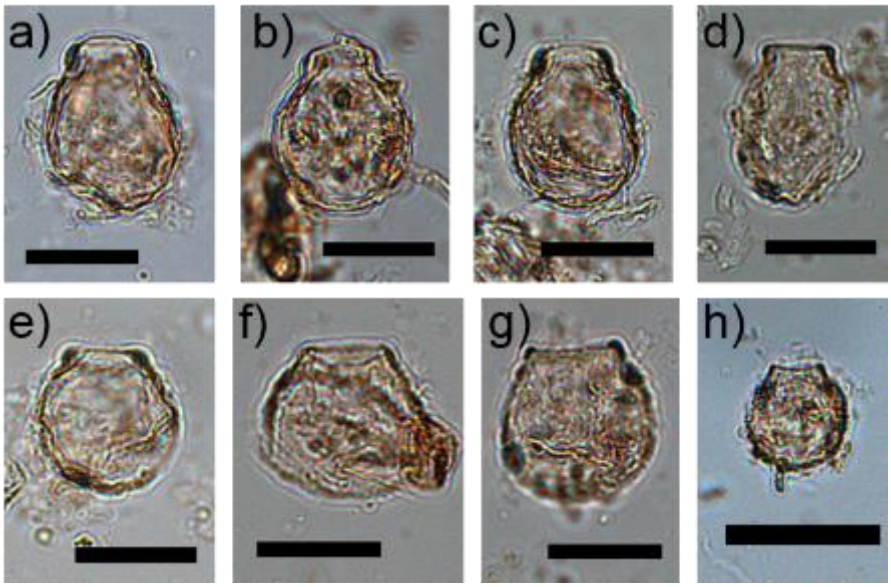

Figure 15 | *Pseudodifflugia spp. 1* (a-d), and ***Pseudodifflugia spp. 2*** (e-h). Scale bar is 20 µm.

Py.spp.1 ***Pyxidicula spp. 1*** (Figure. 16a-f)

Identification notes:

The test is hemispheric or domed in cross-section and circular in lateral view. It appears to be constructed with particles embedded in the test, which gives a rough, untidy appearance, and a patterned surface (Figure 16a). It is always reddish-brown in colour.

Relative abundance: 1.60%

Dimensions:

Diameter:	68 µm (39-76)
Width:	38 µm (37-39)

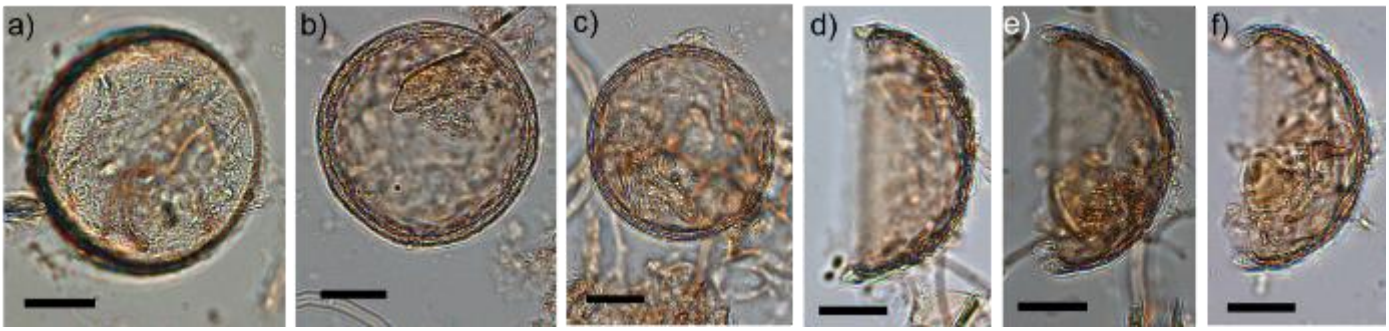

Figure 16 | *Pyxidicula spp. 1* in lateral view (a-c) and cross-section (d-f). Scale bar is 20 µm in all images.

Py.op. ***Pyxidicula operculata*** Agardh 1827 (Figure. 17a-d)

Identification notes:

The test has a distorted circular shape in lateral view, with a patterned border section around the circumference. It appears ephemeral and is transparent to light brown in colour. In cross-section it has a distinctive bowl-shape with steep sides and a flat bottom. Distinctive lobes (Figure 17b,d) are visible in cross-section.

Relative abundance: 0.12%

Dimensions:

Diameter:	37 µm (34-40)
Width:	19 µm (16-21)
Aperture width:	8 µm

Literature Dimensions:

Diameter:	13.6-31 µm	(Microworld 2016)

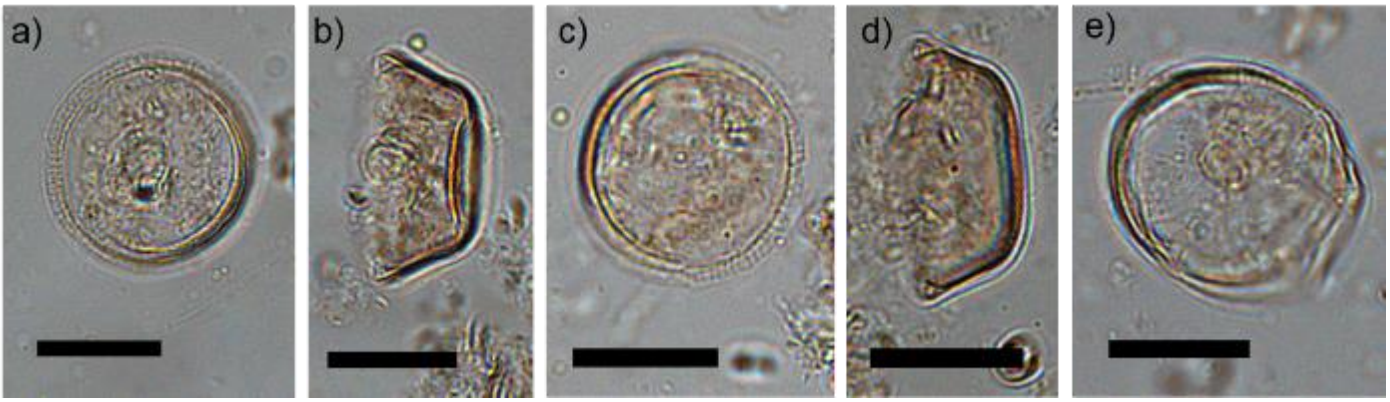

Figure 17 | ***Pyxidicula operculata*** in lateral view (a,c,e) and cross-section (b,d). Scale bar is 20 µm in all images.

Mi.pa. ***Microchlamys patella*** Clararède & Lachmann 1859 (Figure. 18a-e)

Identification notes:

The test is very thin, circular and appears fragile. Its shape is strongly concave or domed in cross-section. A fabric-like, fine membrane is visible on some individuals (Figure 18b) and appears to constrict to a central aperture-like structure in lateral view (Figure 18a).

Relative abundance: 4.88%

Dimensions:

Diameter:	47 µm (34-70)
Width:	29 µm (17-42)

Literature Dimensions:

Diameter:	36-55 µm	(Microworld 2016)

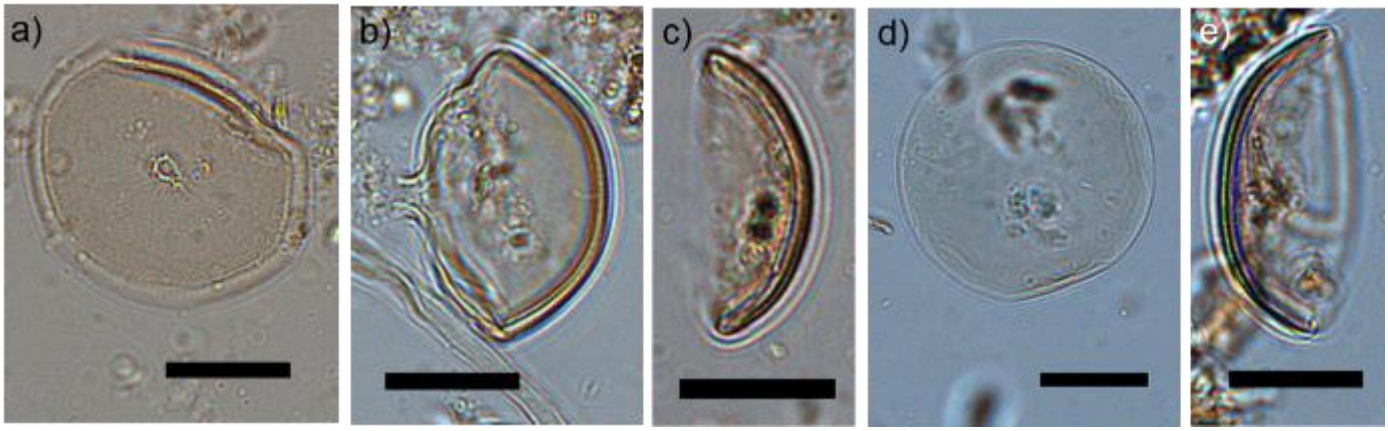

Figure 18 | ***Microchlamys patella*** in lateral view (a, d) and cross-section (b,c,e). Scale bar is 20 µm in all images.

Qu.sy. ***Quadrulella symmetrica**** Wallich 1863 (Figure. 19a-c)

Identification notes:

The test is pyriform, with a relatively wide slightly-convex aperture that is surrounded by a smooth lip. It is composed of characteristic siliceous square plates that are colourless, tight fitting but arranged irregularly across the surface. Plates are generally larger at the posterior end of the test, and smaller around the aperture (Figure 19b).

Relative abundance: 4.08%

Dimensions:

Length:	71 µm (45-90)
Width:	41 µm (33-63)
Aperture width:	18 µm (14-24)
Width/Length:	1:1.71

Literature Dimensions:

Length:	72-103 µm	(Ogden & Hedley 1980)
Width:	36-52 µm	
Depth:	27-35 µm	
Aperture width:	18-22 µm	

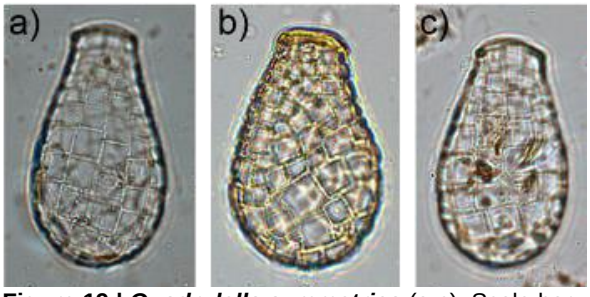

Figure 19 | ***Quadrulella symmetrica*** (a-c). Scale bar is 20 µm.

Tr.de. ***Tracheleuglypha dentata*** Deflandre 1928 (Figure. 20a-b)

Identification notes:

The surface of the test is neatly covered in large circular plates which overlap slightly. Test is transparent, and the aperture is surrounded by a rough collar that is not toothed and therefore helps to differentiate it from *Euglypha rotunda*. It is elliptical in lateral view.

Relative abundance: 1.04%

Dimensions:

Length:	46 µm (39-57)
Width:	26 µm (22-30)
Aperture width:	9 µm (8-12)
Width/Length:	1:1.74

Tr.en. ***Trinema enchelys*** Ehrenberg 1838 (Figure. 20c-d)

Identification notes:

The test is generally ovoid and tapers to a slight point at the posterior end, however individuals frequently appear slightly distorted. It is oval in cross-section. Aperture is circular, and sub-terminal. Primarily differentiated from *Trinema lineare* in this study by its larger size.

Relative abundance: 1.04%

Dimensions:

Length:	43 µm (24-66)
Width:	19 µm (11-34)
Aperture width:	9 µm (5-15)
Width/Length:	1:2.25

Literature Dimensions:

Length:	30-100 µm **M** 47-78 µm **O**	M **=** (Microworld 2016) **O =** (Ogden & Hedley 1980)
Width:	15-60 µm **M** 19-34 µm **O**	
Aperture width:	11-20 µm **O**	

Tr. li. ***Trinema lineare*** Penard 1890 (Figure. 20h-i)

Identification notes:

A smaller version of *Trinema enchelys.* The test is slightly more bulbous at the posterior end compared with *Tr.en.* and does not taper to a point in the same way. We used test length as the primary differentiating variable in this study.

Relative abundance: 0.80%

Dimensions:

Length:	31 µm (23-44)
Width:	13 µm (10-18)
Aperture width:	7 µm (3-12)
Width/Length:	1:2.33

Va.el. ***Valkonovia elegans*** (Figure. 20e-g)

Identification notes:

The test appears similar to *Eu.ro.* but does not taper towards the aperture. It is composed of a large number of small transparent plates that are neatly arranged across the entire surface. Aperture is terminal and very small compared to the test. Overlapping plates sometimes give the aperture a toothed appearance (Figure 20f).

Relative abundance: 0.56%

Dimensions:

Length:	44 µm (33-56)
Width:	29 µm (22-38)
Aperture width:	9 µm (7-13)
Width/Length:	1:1.51

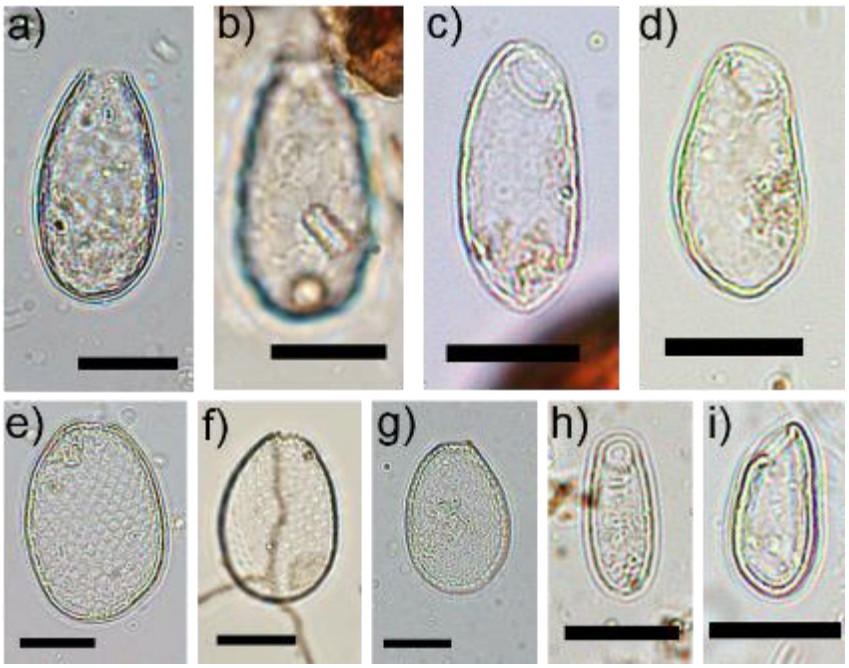

Figure 20 |*Traceleuglypha dentata* (a), ***Traceleuglypha dentata*** detail of plate arrangement (b), ***Trienma enchyls*** (c-d), ***Valkonovia elegans*** (e-g), ***Trinema lineare*** (h-i). Scale bar is 20 µm.

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